CompassCDS
Photodiode Array Software
User Guide

Scion Instruments
1 Bain Square
Livingston, EH547DQ
United Kingdom

©Scion Instruments 2017
Publication Number BR501610
Revision B

Contents

Introduction 3
General 3
PDA Features 3
Acquisition and baseline monitoring 3
Purity calculations 3
Getting Started 4
Configuring the System 4
Starting an Acquisition 4
Building a Method 4
Defining Method Parameters 4
Starting an Acquisition 5
How to View an Acquisition 5
Viewing an Acquisition 5
Viewing a Chromatogram 7
Application Details 8
PDA Data Structure 8
PDA Method and Acquisition 9
The 3D Method 9
The PDA Sub Method 10
The Report Sub Method 11
The Extracted Chromatogram Method 12
The 2D-Signal Chromatogram Method 12
The PDA Specific Variables 12
Baseline Monitoring and Acquisition 14
Following Acquisition 14
First tab (System name + PDA): 15
The Acquisition View Pop-up Menus 17
The Acquisition View Properties 20
Displaying PDA Data 25
Opening a 3-D Chromatographic Data File 25
PDA Chromatogram Display Mode 26
Modifying the display properties 29
The 3D View 34
Displaying Chromatograms and Spectra 36
Getting Chromatograms and Spectra 36
The ASCII Export of Spectra 37
The Spectra Report Editor 38
PDA Data Processing 41
The Spectral Library Editor 41
The ASCII Import of Spectra 44
The CompassCDS ASCII format of spectra 44
The Spectral Library Report Editor 45
The Library Search 48
The Manual Search 48
The Peak Purity 54
The Matching Algorithms of CompassCDS 60
The Spectrum Calculator 61
Reprocess the PDA Chromatogram 62
Index 65

Introduction

General

In conventional HPLC, acquisition of a single chromatogram monitored at a certain wavelength may include many unresolved peaks.

The use of a photodiode array detector adds a third dimension to the chromatogram: the wavelength. The PDA application is a part of the standard CompassCDS Software. In addition to the classical features, the PDA part of CompassCDS is designed to handle this more complex data structure and allows you specific treatment such as:

- determining the optimal wavelength for each peak,
- doing a qualitative analysis using a library search,
- calculating peak purity.

PDA Features

Acquisition and baseline monitoring

- Data display during acquisition and already acquired data display. The 3D data is displayed in real time during the acquisition.
- Qualitative analysis

Purity calculations

- Spectral Comparison at different peak widths
- Spectral Comparison for all points of the peak
- Identification by a library search
- Report display and print

You will rapidly adapt to the PDA environment: in addition to the classical menu at the top of the screen, there are also pop-up menus inside your chromatogram, your spectrum, your isocurve, and your 3D view.

Getting Started

Configuring the System

First, you must create a system in the configuration manager (CompassCDS Configuration Manager). Refer to the CompassCDS Configuration Manager User Guide to create your system.

Starting an Acquisition

Building a Method

In the menu, select ***FILE / NEW / NEW METHOD***.

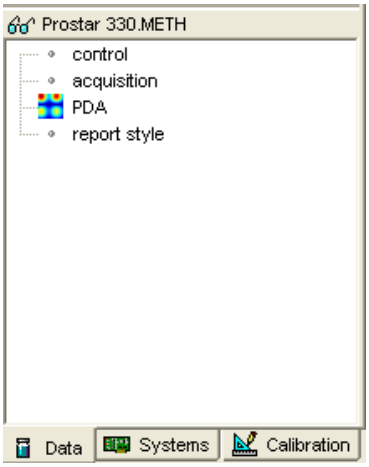
Select the project in which the method will be used (if in all project mode) and the chromatograph on which it will be applied.

Press *Next.*

Enter the name of the method, and optionally, a text description of the method which may also be entered and will appear as a comment in the Open File window.

The method is opened with default parameters in CompassCDS.

To display the parts of the 3D-method, click on the corresponding item in the browser. The parts of the method appear:



Click on the parts of this method to edit the parameters in the right part of the screen.

Defining Method Parameters

In the browser, click on *Control*.

Enter the required instrument method parameters. Refer to the instrument User Guide for more details.

In the browser, click on *Acquisition*.

In this section, parameters may be set that will appear by default in the acquisition windows. For example, the run name can be entered (the identifier or suffix will be implemented automatically for each run).

In the browser, click on *PDA.*

In this section, wavelengths can be defined to automatically extract chromatograms at the end of the run. A sub section corresponding to the chromatogram is automatically added. Processing parameters for each extracted chromatogram can now be defined.

Save the method by using the pull-down menu selections ***FILE / SAVE / SAVE METHOD***, or by clicking on the *Save Method* icon, or by pressing the key combination Shift+Ctrl+2.

Starting an Acquisition

Before starting a baseline monitoring or an acquisition, a method must be prepared for the corresponding system (chromatograph).

It is possible to launch a Quick Start (only one acquisition) or a Sequence of acquisitions. Refer to the CompassCDS User Guide to learn how to start an acquisition.

How to View an Acquisition

Viewing an Acquisition

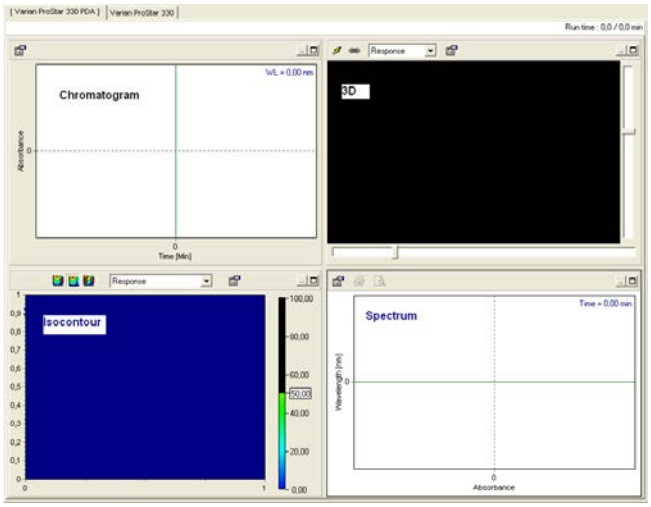
In the CompassCDS main screen, go to the Systems tab.

In the browser, select the system where the sample was injected. Check *PDA* to view the 3D data acquisition. Check *status* to also view the system status. Possibly, check *Chromatogram* to view the 2D signal if any.

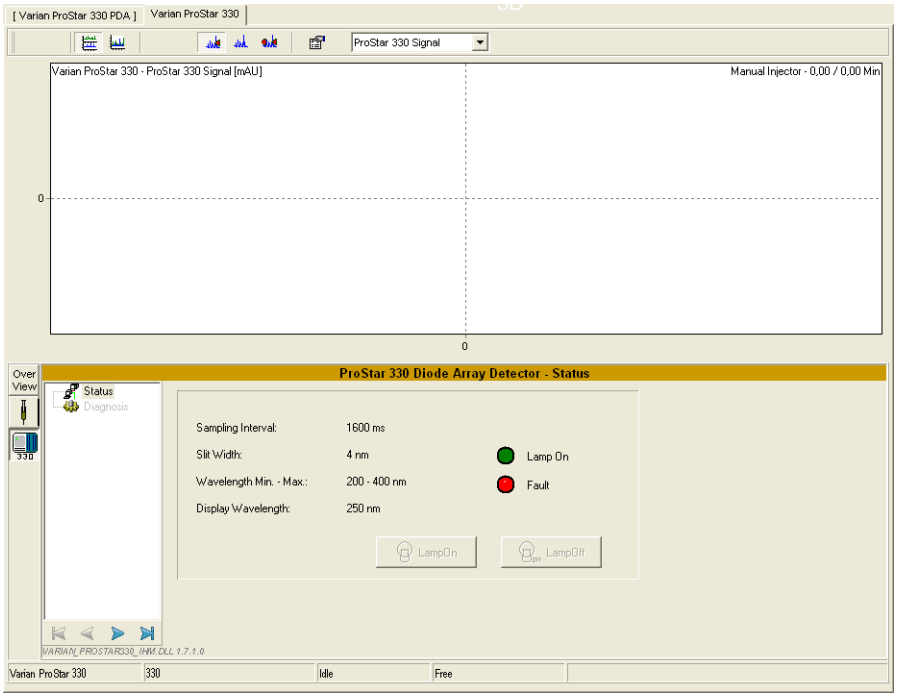
In the right part of the screen, two tabs appear.

The first one, named PDA (preceded by the system name) is composed of 4 views:

- the Chromatogram view
- the 3D view (which is initially empty)
- the Isocontour plot view
- the Spectrum view



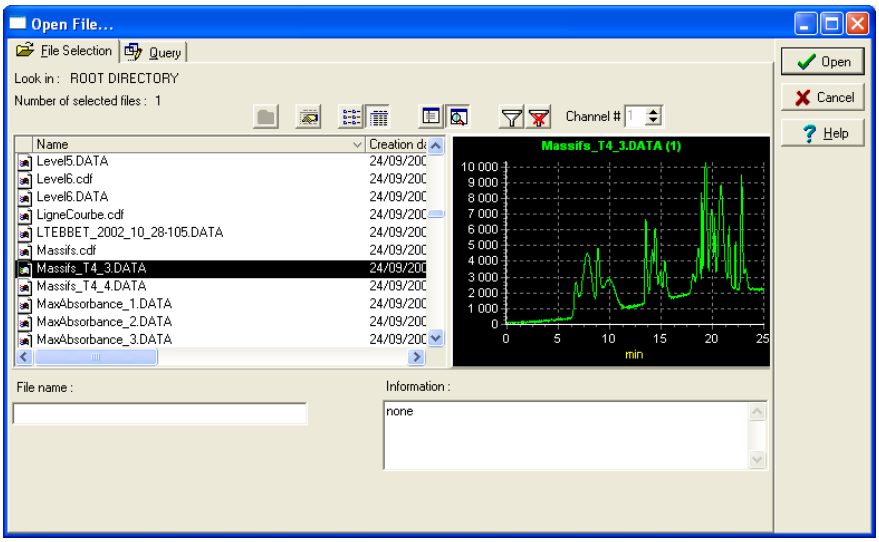
The second tab, named by the system name, contains the system status and possibly the 2D signal(s).



Viewing a Chromatogram

Once the chromatogram has been acquired, open and process it press .

The Open File window appears:

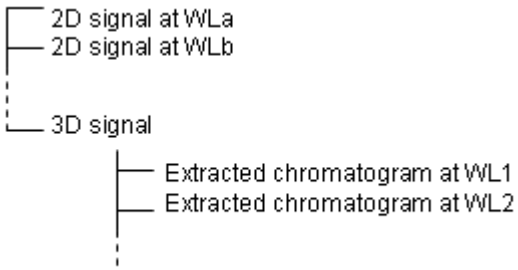


In this window, use the scroll bars to scroll down the list and click on the just acquired chromatogram. Alternately, type the first few letters of the chromatogram name in the file name box and only the chromatograms whose names begin with these letters will be displayed.

Application Details

PDA Data Structure

By default, PDA data consist in one 3D signal and possibly one or several chromatograms (2D signal) acquired at fixed wavelength. Chromatograms can additionally be extracted from the 3D signal. Data structure is as follows:



The 2D signals can be processed as classic chromatograms (integration, peak and group identification, calibration, etc.). From the 3D signal, user can add spectra in libraries or perform manual library search. From the extracted chromatograms, the user can perform all the classical processing of 2D channels and can also calculate peak purity or perform automatic library search.

PDA Method and Acquisition

To launch an acquisition the user must first define a 3D method and optionally, extract chromatogram methods for having chromatograms automatically extracted at the end of the run.

The 3D Method

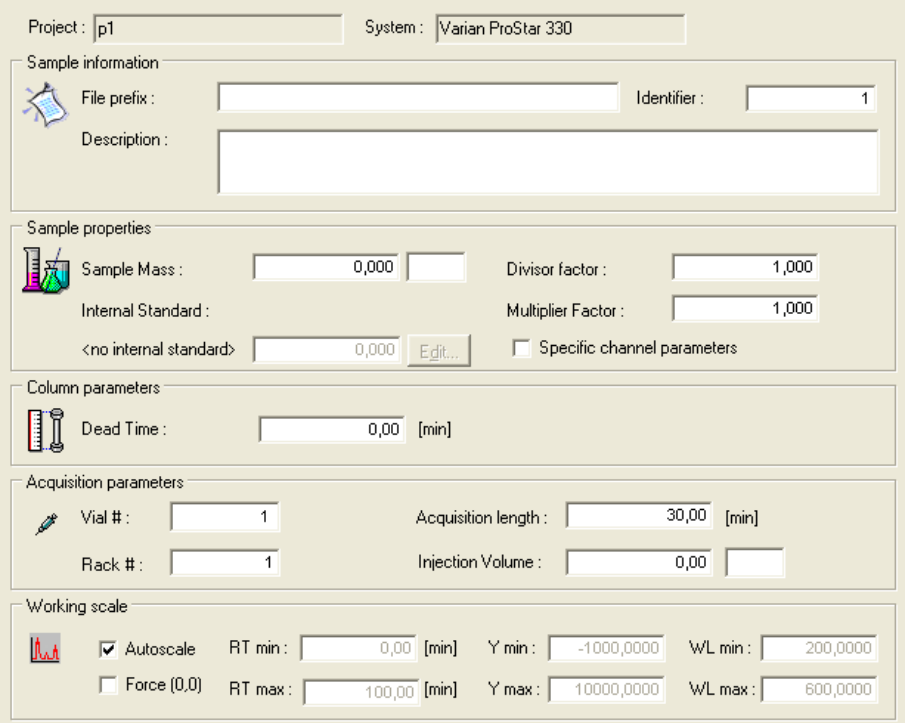
The 3D-method is divided into four parts.

The Control Sub Method

This part of the method depends on the instrument connected to the channel. The control method is composed of all the parameters that can be set for this instrument. Refer to the instrument driver manuals to learn how to fill this section.

The Acquisition Sub Method

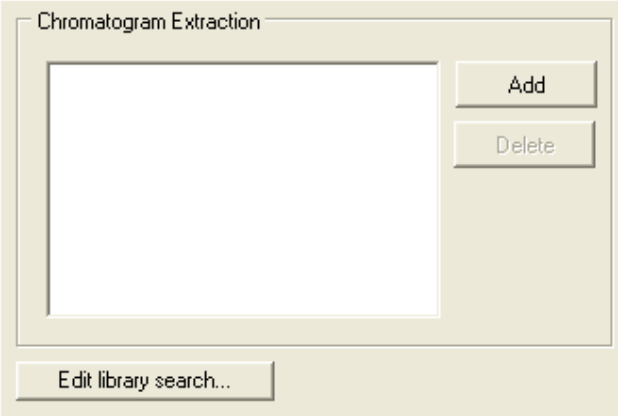
In the acquisition method, default parameters can be set. They will be displayed in the acquisition windows: Quick Start or Sequence.



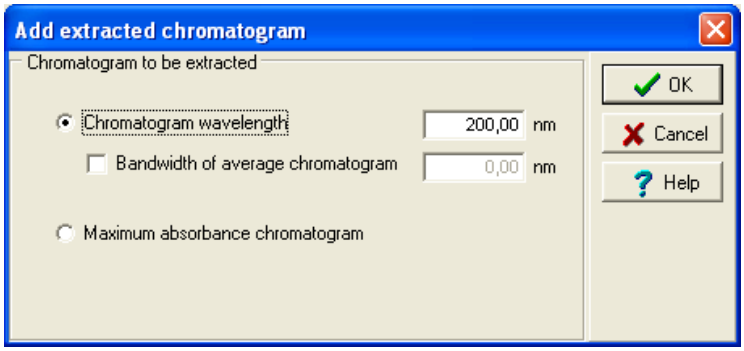
It is possible to modify these parameters before starting the acquisition. Refer to the CompassCDS User Guide for a description of the fields.

The PDA Sub Method

This sub section allows you to select the chromatograms automatically extracted at the end of the acquisition and to define the parameters of automatic library search. The library search is also described on page 48. The following screen is displayed when the PDA sub method is checked.



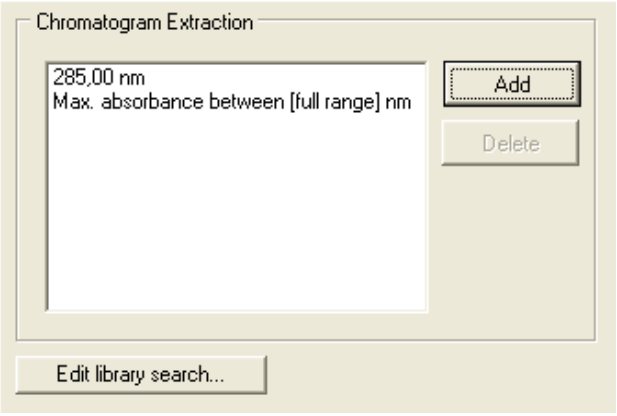
In the Chromatogram Extraction field, you can **add** the chromatogram you want to automatically extract after the acquisition. Click on the *Add* button, to choose the chromatogram you want to extract. The following screen appears:



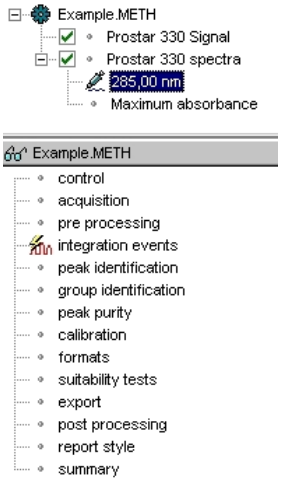
You can choose to extract a chromatogram at either a given wavelength or at an average wavelength over a defined bandwidth, or at the maximum absorbance chromatogram obtained on the full acquisition wavelength range or in a user defined wavelength range.

The maximum absorbance curve is a virtual chromatogram where the wavelengths are chosen so that each chromatogram point is plotted at the optimum wavelength which gives the maximum absorbance.

The following screen gives an example of the extraction of the chromatogram at 285 nm and the maximum absorbance chromatogram.



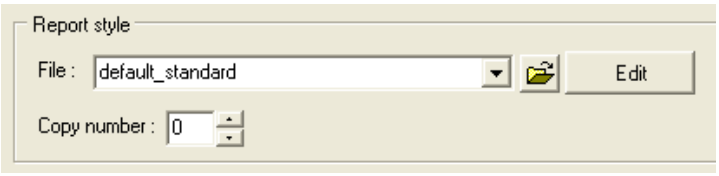
For each extracted chromatogram, a sub-section is created in the browser. Its name corresponds to the wavelength at which the chromatogram is extracted:



An acquisition can be started without specifying an extracted chromatogram.

The Report Sub Method

The **Report Style** sub section allows you to define the PDA report style. The following screen is displayed:



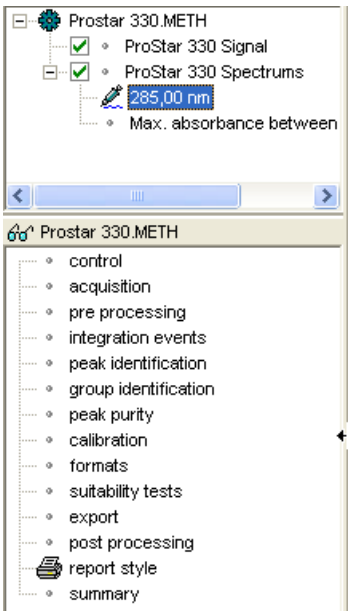
In the **File** box, select the name of the report style to be printed. Styles should first be created in CompassCDS Report Editor (see the corresponding User Guide).

To look at the selected report, press the *Edit* button: the CompassCDS Report Editor is opened and displays the report style.

To print automatically one report after an acquisition, enter 1 in the **copy number** box. If 0 is selected, no printing will be done.

The Extracted Chromatogram Method

A method sub-section is created for each extracted chromatogram. Check the right channel in the browser to modify or display the corresponding method. For example, in the following screen, the method displayed in the browser is the method of the chromatogram extracted at 285 nm.



The **Control** and the **Acquisition** part are the same for the 3D data and for the extracted chromatogram(s).

All the sub parts of the extracted chromatogram(s) are described in details in the CompassCDS User Guide, except the peak purity one.

The peak purity part is described on page 54.

The 2D-Signal Chromatogram Method

The 2D-signal chromatogram method can be handled as a classical method. See the CompassCDS User Guide for a detailed explanation for each method sub-part.

The PDA Specific Variables

CompassCDS PDA incorporates many standard variables that can perform calculations on the entire sample.

It is also possible to define custom variables for calculations that not already belong to the default CompassCDS variables such as:

> “user input”: variables for which the user must enter a value or a text.
>
> “user formula”: variables that allow CompassCDS non default calculations (see the CompassCDS User Guide for more details).

All the PDA-variables (system, user input and user formula) are defined using specific parameters, described in the CompassCDS User Guide.

Most of the Variables present by default in CompassCDS PDA are the same as the ones present in CompassCDS. Only the PDA specific variables are described here. Please refer to the CompassCDS User Guide to have a complete description of other variables.

Variables of the 3D Channel

Only one 3D variable is PDA specific. It is a global variable:

> **Number of WL Points [NWLPOINTS]**: Number of points acquired in the UV spectrum.

Note: The global variables belong to the chromatogram in its whole. The global variables can be displayed in the chromatogram properties. Select the menu options *DATA / CHROMATOGRAM PROPERTIES* in the main menu or press the icon.

Variables of the 2D-signal Channel

No specific PDA variables exist. (refer to the CompassCDS User Guide for more details).

Variables of the Extracted Chromatogram

PDA specific variables exist by default in CompassCDS PDA related to purity and search in library. All these variables are Peak variables.

Note: The peak variables can be displayed in the peak report. (Choose REPORT PROPERTIES in the popup menu and select the variables for report display).

Purity Variables

Area Impure (%) [AREA_IMPURE]: Area percentage of peak computed as impure. Only computed if the purity is calculated for "all spectra" of each peak (see page 54).

Area Medium (%) [AREA_MEDIUM]: Area percentage of peak computed as medium. Only computed if the purity is calculated for all spectra of each peak.

Area Pure (%) [AREA_PURE]: Area percentage of peak computed as pure. Only computed if the purity is calculated for "all spectra" of each peak (see page 54).

Pur(Apex) [PURITY_APEX]: Purity value calculated at peak apex. Computed for all options of spectral purity (see page 54).

Pur(FBL) [PURITY_FB]: Purity value calculated at peak front baseline. Computed for all options of spectral purity except "3 spectra" and "apex only" (see page 54).

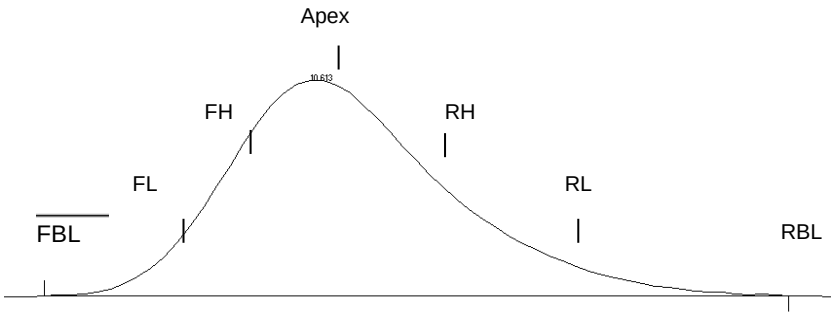
Pur(FH) [PURITY_FH]: Purity value calculated at a peak height defined by the user. Four heights maximum can be defined. This is the front high value. Computed for all options of spectral purity except "apex only" (see page 54).

Pur(FL) [PURITY_FL]: Purity value calculated at a peak height defined by the user. Four heights maximum can be defined. This is the front low value. Computed for all options of spectral purity except "3 spectra" and "apex only" (see page 54).

Pur(RBL) [PURITY_RB]: Purity value calculated at peak rear baseline. Computed for all options of spectral purity except "3 spectra" and "apex only" (see page 54).

Pur(RH) [PURITY_RH]: Purity value calculated at a peak height defined by the user. Four heights maximum can be defined. This is the rear high value. Computed for all options of spectral purity except "apex only" (see page 54).

Pur(RL) [PURITY_RL]: Purity value calculated at a peak height defined by the user. Four heights maximum can be defined. This is the rear low value. Computed for all options of spectral purity except "3 spectra" and "apex only" (see page 54).



Search in Libraries Variables

Matching Component [MATCHCOMPO]: name of the component which better matches the unknown spectra.

Matching Factor [MATCHFACTOR]: matching factor corresponding to the component which better matches the unknown spectra.

Matching Library [MATCHLIBRARY]: Spectra library of the component which better matches the unknown spectra.

Others

WL(MaxAbs) [WL_MAXABS]: wavelength at the maximum absorbance of the peak.

Baseline Monitoring and Acquisition

Before starting a baseline monitoring or an acquisition (Quick Start or sequence), a 3D-method must be prepared for the corresponding system (chromatograph).

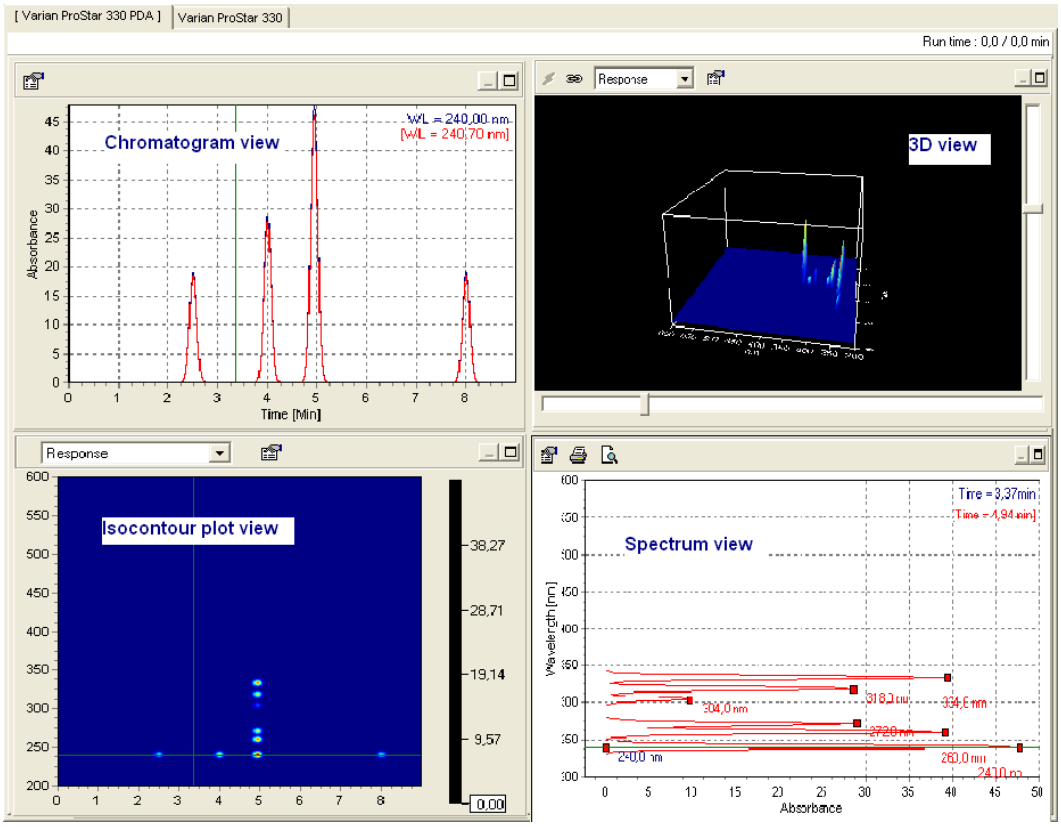
See the CompassCDS User Guide on how to launch a baseline monitoring, a Quick Start (injection of one sample) or a sequence (injection of several samples).

Following Acquisition

To look at a PDA chromatogram during its acquisition, select the **Systems** tab. All the systems defined in the project in which the user is connected, are listed in the browser.

If a PDA system name is checked, two tabs are created: one named by the system name following with PDA, the other named by the system name only.

First tab (System name + PDA):



By default, the screen is composed of four parts:

- the Chromatogram view
- the 3D view (which is initially empty)
- the Isocontour plot view
- the Spectrum view

Use the following icons or the scrolling list to:

Stop the current run. If the instrument is fully controlled, CompassCDS does not receive any information that the instrument is stopped. It is therefore necessary to stop the run within the software. CompassCDS will ask for a confirmation before stopping a run. Only the person who started an acquisition, or those users defined in the CompassCDS Configuration manager have the right to stop it. In the case of double injection acquisition, user can choose to stop either one or both acquisitions.

Modify the acquisition time. Note that by decreasing the acquisition time to a value less than the current run time, the acquisition will be stopped.

Display the views with automatic scaling: the scales are updated after each acquisition point, so the entire chromatogram is displayed.

Display the views in a fixed window. The parameters can be changed in the properties.

Set the parameters for the x-scale that moves along as the acquisition runs. For example, it is possible to view only the last minute of acquisition. To modify the width of this window, use the properties (see the corresponding icon just below) and in the scrolling section, it is possible to change this default value.

Modify the acquisition view properties, see page 20.

Select which response you want to display. This scrolling list is present in both isocontour map and 3D view. Four choices are proposed: Response, Threshold up, Threshold down and Threshold between to display respectively all the absorbance levels with the same importance or emphasize certain ones. All absorbances inferior to the lower limit or superior to the upper limit will have the same color.

Create the 3D view. By default, the 3D view is empty.

Synchronize the zoom between the views.

Maximize the corresponding view to display it in the full area dedicated to acquisition views

Minimize the corresponding view size and return in the 4 views mode.

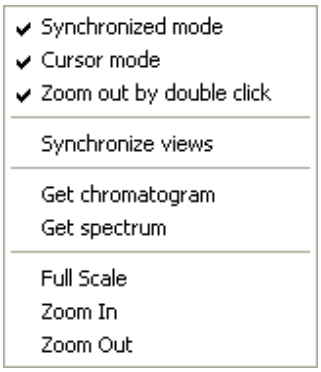
Print a spectra report

Print preview a spectra report

The Acquisition View Pop-up Menus

In each of the views, pop-up menus accessible by mouse right-click provide access to multiple functions.

The Isocontour Plots View Pop-up Menu



If the **Synchronized mode** is checked, the views are always synchronized: zoom done in one view is always applied in other views.

If the **Cursor mode** is checked, when you move the cursor using the mouse in the isoabsorbance map, the corresponding chromatogram and spectrum are displayed in the corresponding views. The time and wavelength position markers are moved accordingly in each view. When a point is correctly chosen, you can deactivate the cursor mode (by unchecking it in the pop-up menu). The chromatogram and the spectrum do not change regardless of the mouse movement in the isocontour map except after a left mouse double-click.

If **Zoom out by double click** is checked, the last zoom is applied after each double clicks.

Click on the **Synchronize views** to apply punctually the same zoom in all the views if synchronized mode is unchecked.

Click on the **Get chromatogram** option to fix one chromatogram at the current wavelength.

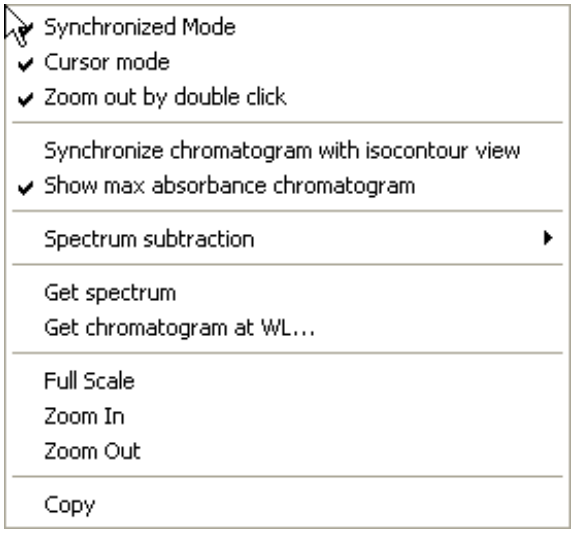
Click on the **Get spectrum** option to fix one spectrum at the current time.

Click on **Full Scale** to return to the full scale.

Click on **Zoom In** to magnify the view.

Click on **Zoom Out** to reduce the view.

The Chromatogram View Pop-up Menu



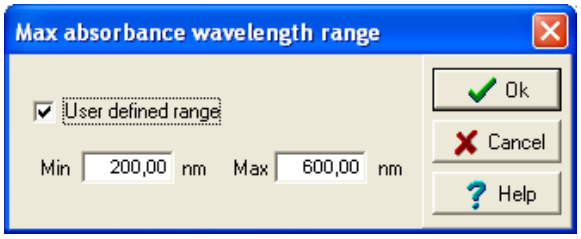
If the **Synchronized mode** is checked, the views are always synchronized: zoom done in one view is always applied in other views.

If the **Cursor mode** is checked, when you move the cursor in the views using the mouse, the time position marker is moved in the chromatogram and isocontour views and the corresponding spectrum is displayed in the spectrum view. When a point is correctly chosen, you can deactivate the cursor mode (by unchecking it in the pop-up menu). The time repair does not move and the spectrum does not change regardless of the mouse movement in the chromatogram view except after a left mouse double click.

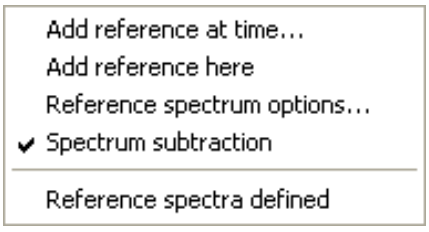
If **Zoom out by double click** is checked, the last zoom is applied after each double clicks.

Click on the **Synchronize chromatogram with isocontour view** option to punctually apply the isocontour view zoom on the chromatogram when synchronized mode is unchecked.

Click on **Show max absorbance chromatogram** to display the maximum absorbance chromatogram which shows all components at their maximum absorbance. The following screen appears:



Click on the **Spectrum subtraction** option to select a spectrum to subtract. The following screen appears:



Choose **Add reference at Time** and enter manually a time where a reference spectrum will be defined in the appearing screen.

Click on **Add reference here** to define a reference spectrum at the cursor time.

Choose **reference spectrum options** to remove some reference spectra.

Check **Spectrum subtraction** to activate or not the subtraction. A spectrum is interpolated between two consecutive reference spectra and subtracted to all spectra of the corresponding interval. The results of the subtraction are given in the spectrum view.

When reference spectra have been defined, "Reference spectra defined" appears in the bottom part of that popup menu. Else, "No reference spectrum defined" appears.

Click on **Get Spectrum** to fix one spectrum at the current time.

Click on **Get Chromatogram at WL** to enter manually a wavelength and get the corresponding chromatogram.

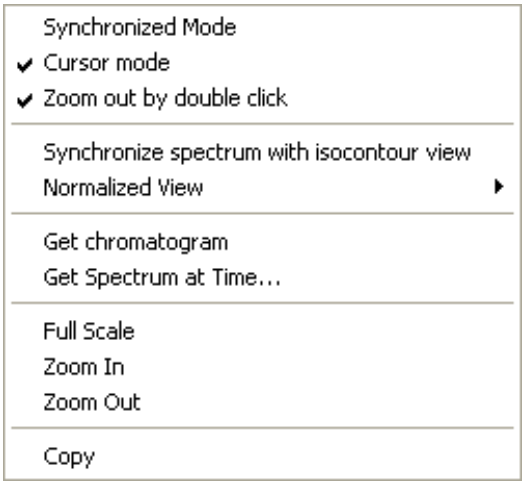
Click on **Full Scale** to return to the full scale.

Click on **Zoom In** to magnify the view.

Click on **Zoom Out** to reduce the view.

Click on **Copy** to copy the chromatogram as displayed and paste it to another application.

The Spectrum View Pop-up Menu



If the **Synchronized mode** is checked, the views are always synchronized: zoom done in one view is always applied in other views.

If the **Cursor mode** is checked, when you move the cursor in the view using the mouse, the wavelength position marker is moved and the corresponding chromatogram is displayed in the corresponding view. When a point is correctly chosen, you can deactivate the cursor mode (by unchecking it in the pop-up menu). The wavelength marker and the corresponding chromatogram the do not change whatever the mouse movement in the spectrum view except after a left-mouse double click.

If **Zoom out by double click** is checked, the last zoom is applied after each double click.

Click on **Synchronize with isocontour view** to punctually apply the isocontour view zoom on the spectrum when synchronized mode is unchecked.

Click on **Normalized View** to apply a specific scale on the absorbance axis. A submenu is displayed with two choices (**auto** and **at WL**). Depending upon the chosen option, the spectra are zoomed in such a way that the signal corresponding to the maximum

absorbance (auto mode) or the absorbance at the specified wavelength (at WL mode) represents 5 to 95% of the absorbance axis.

Click on the **Get chromatogram** option to fix one chromatogram at the current wavelength.

Click on **Get spectrum at time** to enter manually a time and get the corresponding spectrum.

Click on **Full Scale** to return to the full scale.

Click on **Zoom In** to magnify the view.

Click on **Zoom Out** to reduce the view.

Click on **Copy** to copy the chromatogram as displayed and paste it to another application.

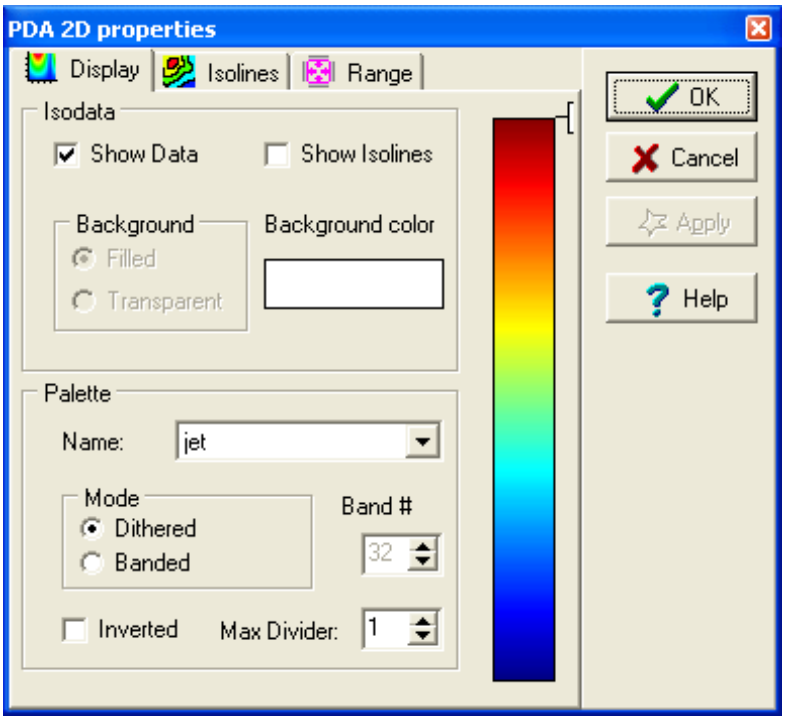
The Acquisition View Properties

Click on the button in each view to change its display properties.

The Isocontour View Properties

Three tabs allow you to modify the acquisition displaying properties: Display, Isolines and Range.

Display



In this tab customize the isocontour view display.

Check or uncheck **show data** to display or hide the acquisition points when *show isolines* is checked. If **show data** is unchecked, only the isolines are displayed.

Check or uncheck **show isolines** to display or hide the isolines.

If *show data* is unchecked, you can change the background color. Click on the mouse left button in the **background color** field and choose a color in the appearing screen. It is also possible to make the background transparent and display the scale grid (select **transparent** in the background field).

In the **palette** field customize the color scale of the view. The resulting scale is displayed on the screen right column. Change the **name** to modify the absorbance-color correspondence. It is also possible to invert the scale (check **inverted**) or to emphasize certain absorbances by changing the value of the **Max Divider** (see below for more explanation). The coloration can also be **dithered** or **banded**. In this case you can choose the number of bands.

What does the Max Divider Parameter mean?

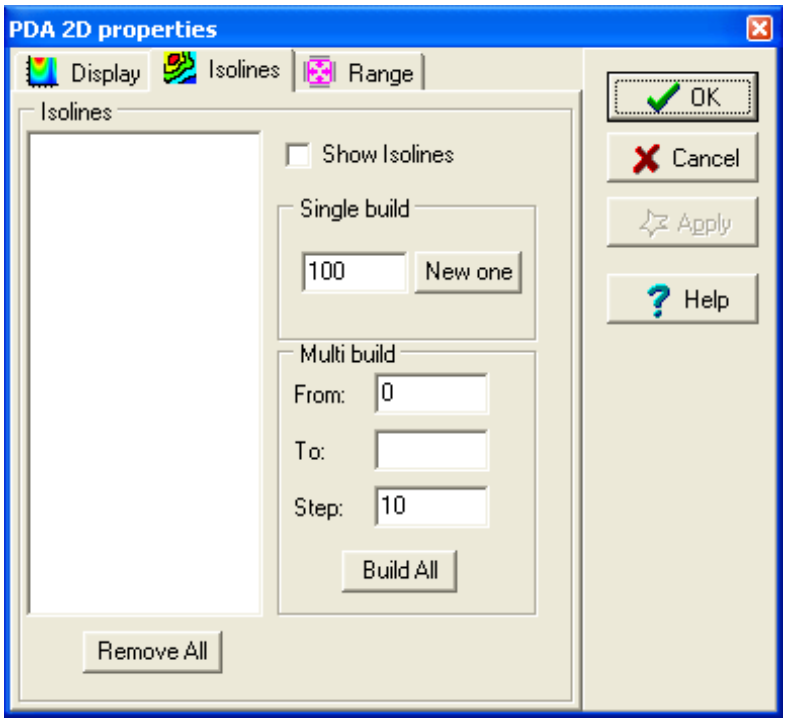
The Max Divider Parameter will define the limit of color levels displaying the isocontour view.

The inferior limit of the color level 7 (level for the largest absorbance values) will be divided by the max divider parameter. As a consequence, if Max Divider is superior to one, this level range will be larger and will contain more data points: above a certain value of absorbance, the data will be displayed with the same color (the color chosen for level 7).

The other color level range (1 to 6) will be narrowest. As a consequence, the small variations will be emphasized on the isocontour view.

As a conclusion, if you want to emphasize the small variations of the isocontour view, choose a large value.

Isolines



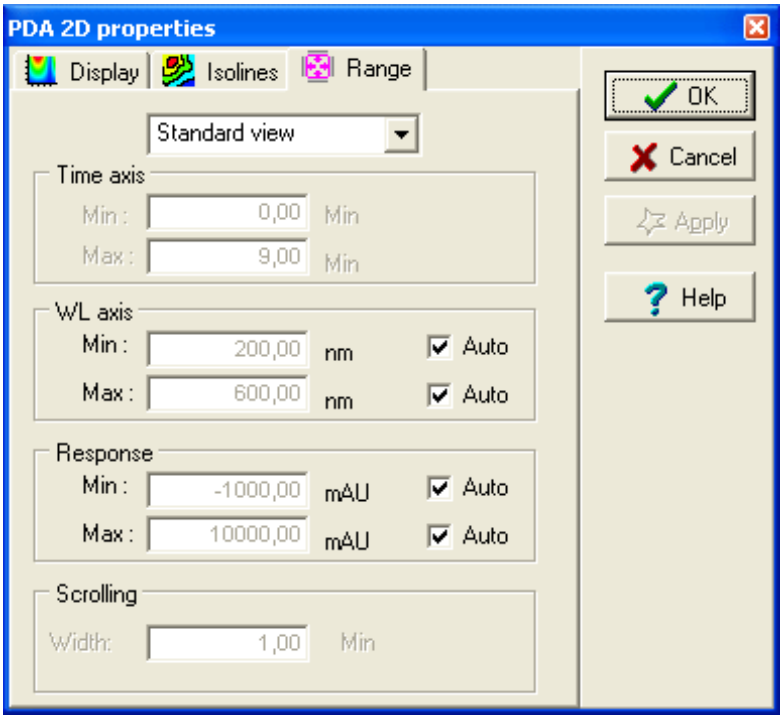
In the **Isoline** tab, check **Show Isolines** if you want to display isolines.

Choose the absorbance line you want to display in the single build field and then click on *New one.*

To automatically build the isolines, choose in the **Multi build** field, the minimum absorbance (From: field), the maximum absorbance (To: field) and the Step. Then click on *Build All.*

After choosing the isolines, their absorbance values are given in the Isolines field. You can uncheck the isolines you do not want to display.

Range

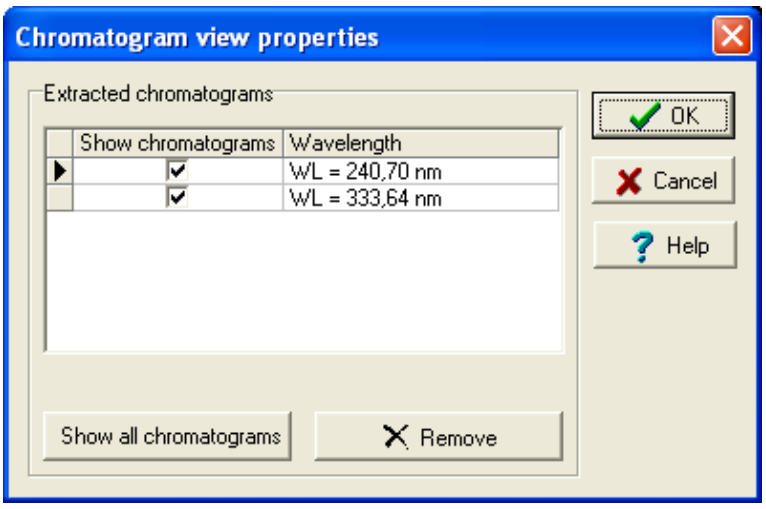


In the **Range** tab, you can choose the acquisition view properties (standard, fixed, or scrolling view) using the scrolling list. If the standard view is chosen, you can modify the Wavelength and the Response axis range. If the fixed view is chosen, moreover, the Time axis range can be modified. If the scrolling view is chosen, the scrolling width must be fixed in the field.

An automatic scale means that the minimum is set to the minimum signal value and the maximum is set to the maximum signal value: the response and the WL-scale are large enough to display all the acquisition points.

The Chromatogram View Properties

The chromatogram view properties allows the user to manage the chromatogram to display. The screen looks as follows:



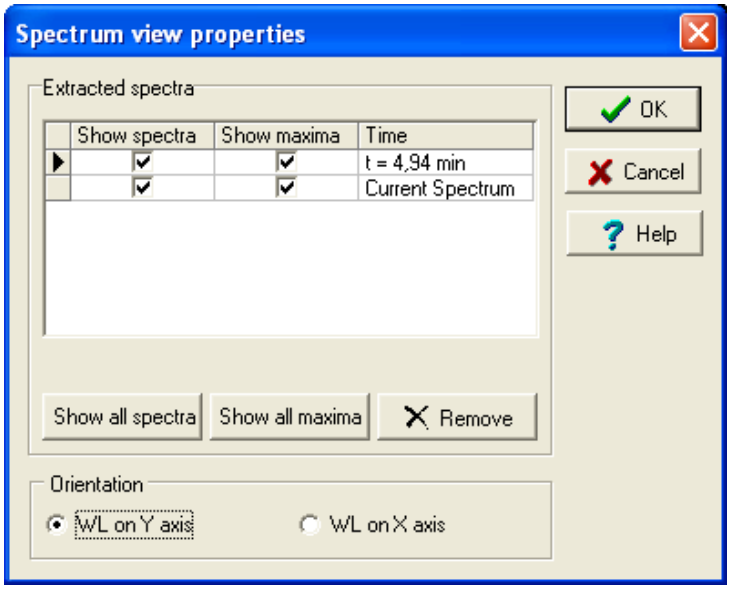
Choose which chromatogram(s) you want to display by checking the corresponding box.

Press the *Remove* button to delete chromatogram(s) you do not want to display.

Press the *Show all chromatograms* button to display all the listed chromatograms.

The Spectrum View Properties

The spectrum view properties allow you to manage the spectra to display. The screen looks as follows:



Choose which spectrum(a) you want to display by checking the corresponding box in the *Show spectra* column.

Choose which spectrum maximum(a) you want to display by checking the corresponding box in the *Show maxima* column. You can also choose to hide/show given maximum(a) by clicking on their box directly in the spectrum view.

Press the *Show all spectra* button to display all the listed spectra.

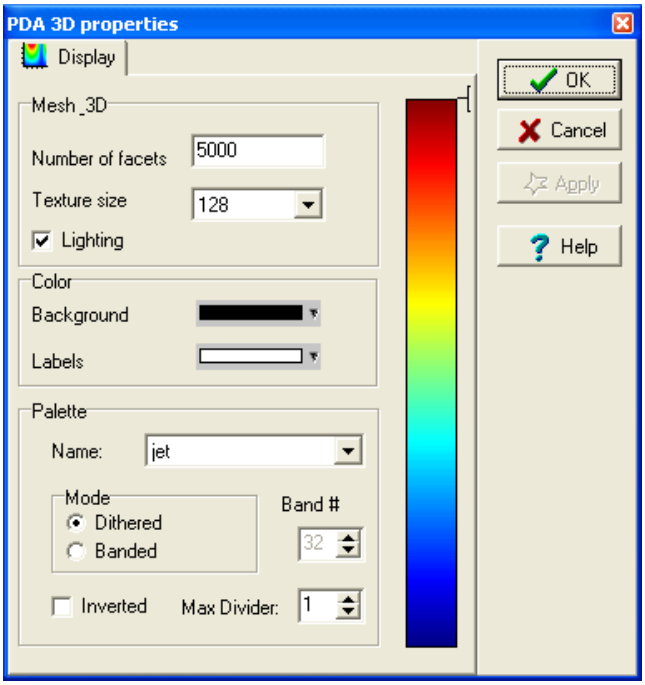
Press the *Show all maxima* button to display the maxima of all the selected spectra.

Press the *Remove* button to delete spectrum(a) you do not want to display.

Select the *WL on Y axis* or *WL on X axis option* to display the spectrum vertically or horizontally.

The 3D View Properties

The following screen allows you to modify the acquisition 3D displaying properties:



You can modify the appearance of the 3D object:

The number of facets and the texture size can be modified in the Mesh-3D field.

The color of the background and the labels can be chosen in the color field.

In the **palette** field customize the color scale of the view. The resulting scale is displayed on the screen right column. Change the **name** to modify the absorbance-color correspondence. It is also possible to invert the scale (check **inverted**) or to emphasize certain absorbance by changing the value of the **Max Divider** (see below for more explanation). The coloration can also be **dithered** or **banded**. In this case you can choose the number of bands.

What does the Max Divider Parameter mean?

The Max Divider Parameter will define the limit of color levels displaying the 3D view.

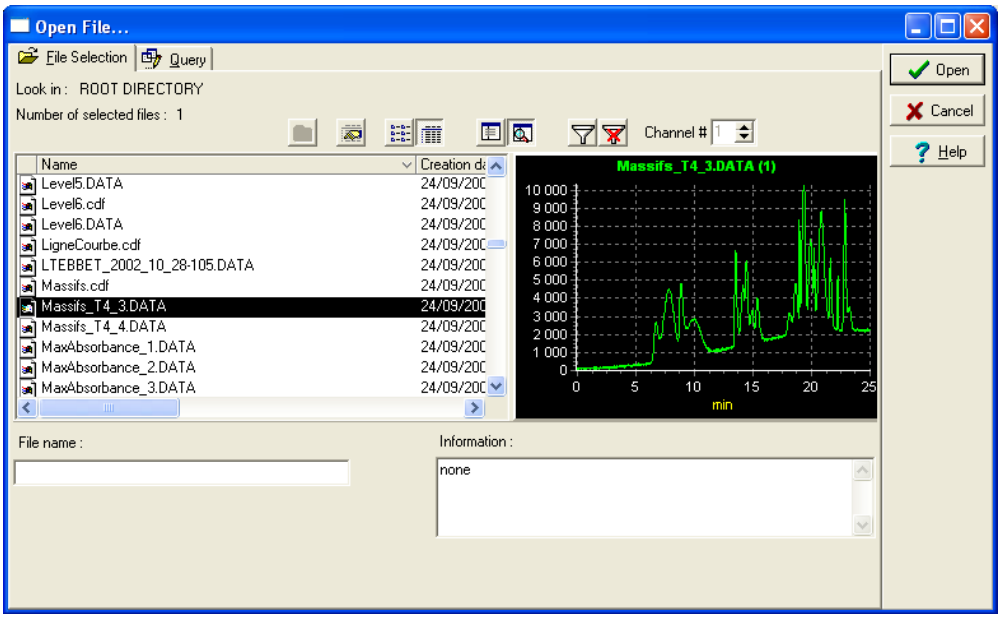
The inferior limit of the color level 7 (level for the largest absorbance values) will be divided by the max divider parameter. As a consequence, if Max Divider is superior to one, this level range will be larger and will contain more data points: above a certain value of absorbance, the data will be displayed with the same color (the color chosen for level 7).

The other color level range (1 to 6) will be narrowest. As a consequence, the small variations will be emphasized on the 3D View. As a conclusion, if you want to emphasize the small variations of the 3D display, choose a large value.

Displaying PDA Data

Opening a 3-D Chromatographic Data File

Select ***FILE / OPEN / OPEN CHROMATOGRAM*** or click on the button or press Ctrl+O. When the Open File dialog box appears, select your file in the File selection tab. Double click on it or select OK. No preview is displayed for the spectrum channel in the right part of the Open File box for PDA chromatograms.

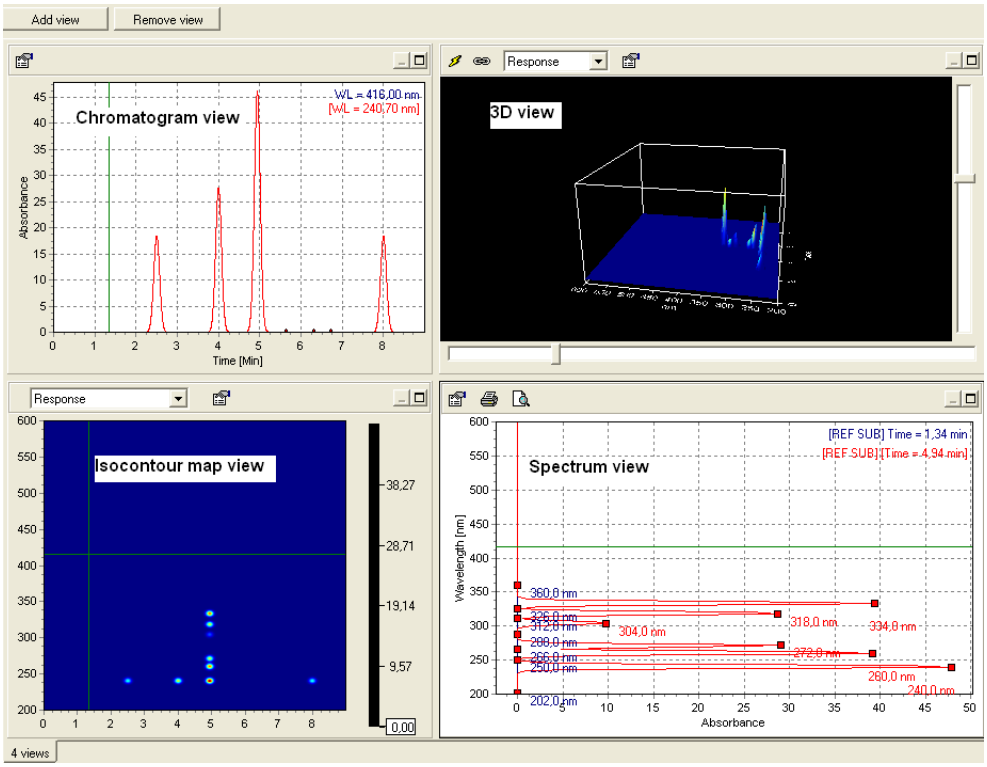


See the CompassCDS User Guide to know how to manage the open file box.

PDA Chromatogram Display Mode

In CompassCDS, four standard display modes exist named '4 views', '3 views', '3D only' and 'Spectral extraction'.

By default, when a PDA chromatogram is opened, the ‘4 views’ is displayed in the right part of the screen and the following screen appears:



The ‘4 views’ consists of:

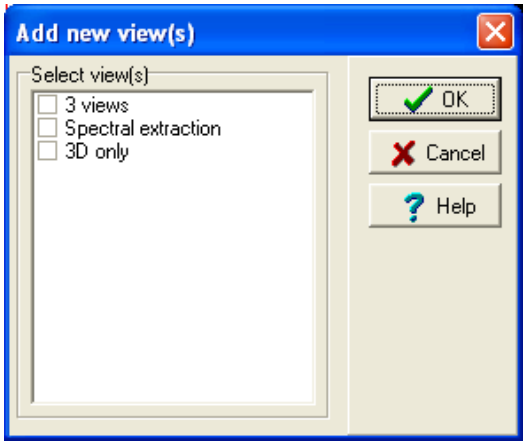
The isocontour view: 3D data displayed in a 2D view. The absorbance levels are represented by different colors, each color corresponding to an absorbance range.

- The 3D view.
- The chromatogram view.
- The spectrum view.

The chromatograms and the spectra displayed correspond to the current cursor position in the isocontour map and user defined chromatograms and spectra (see page 36 for details).

You can add several other views using the Add view button or delete the opened ones using the Remove view button.

When you click on Add view, the following screen appears:

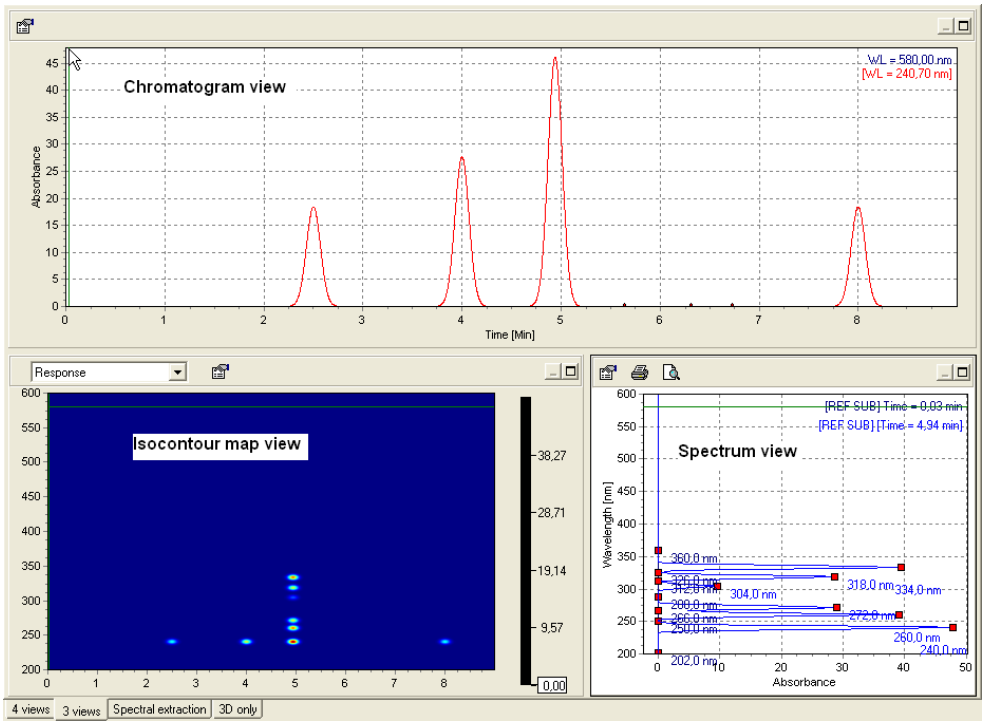


Choose the view(s) you want to display. All the views are linked : if one action is performed in one of the view (get chromatogram, get spectrum or normalization), it will automatically be applied in the other ones.

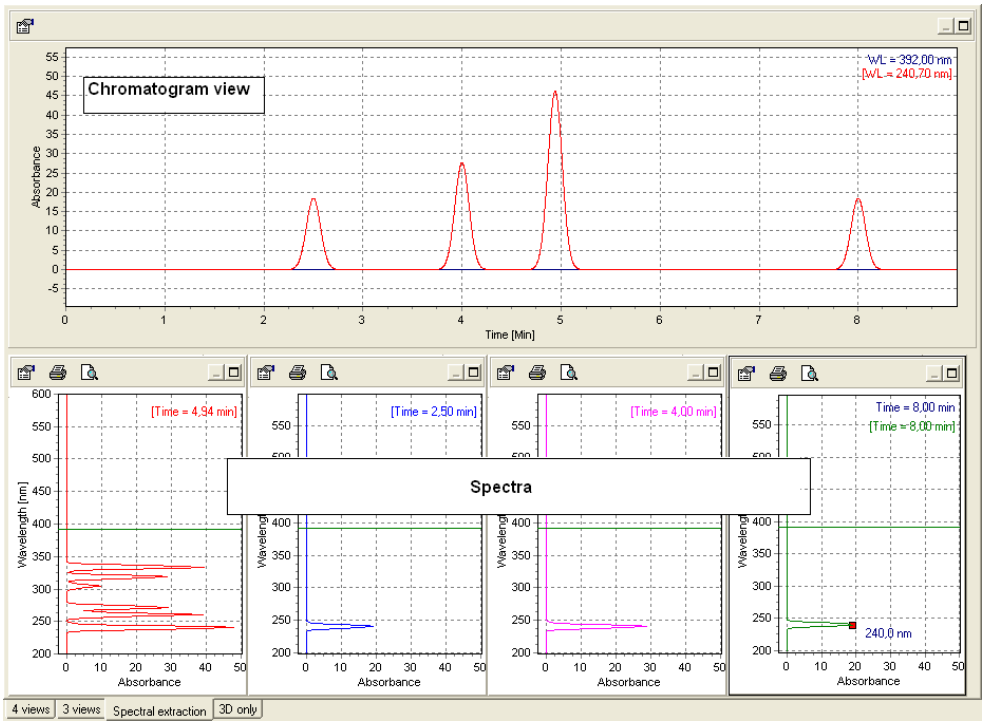
Each view is displayed in full page. Click on the corresponding tab to select the view to display.

The '3 views' consists of:

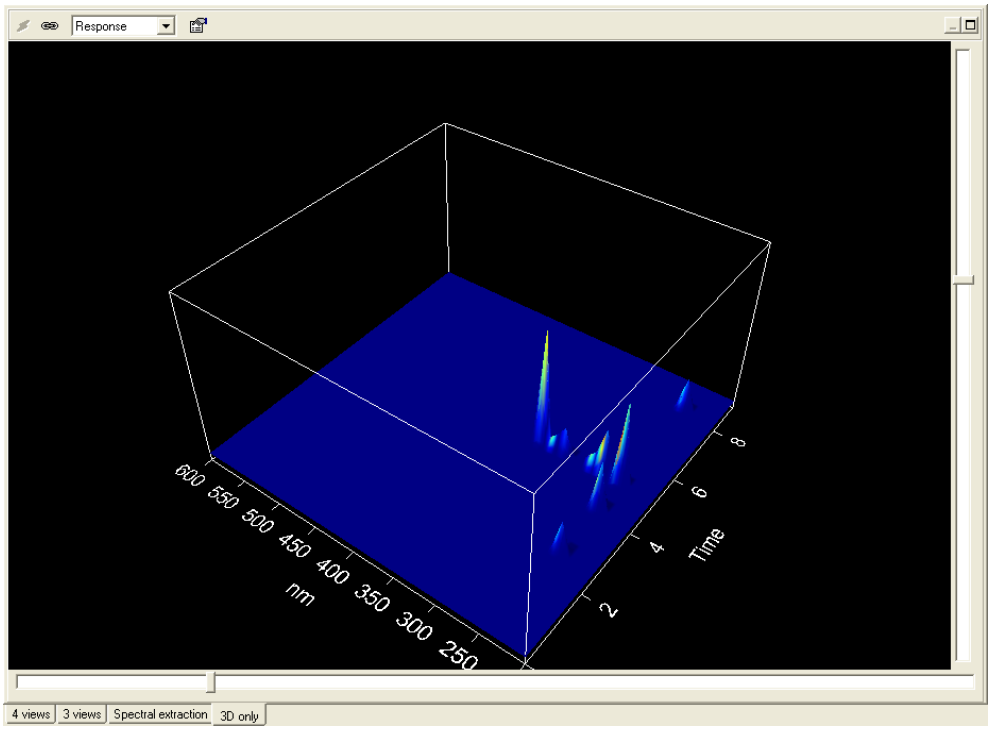
- The spectrum view.
- The chromatogram view.
- The isocontour view.



The 'spectral extraction' view contains one area with the chromatogram(s) and several area(s) (4 by default) with the spectra. The spectra field number can be changed using the spectra field pop-up menus. Select *Change spectra view number* and type the number of fields to be displayed.



The '3D only' view shows the data in a 3D view.



Modifying the display properties

The isocontour View

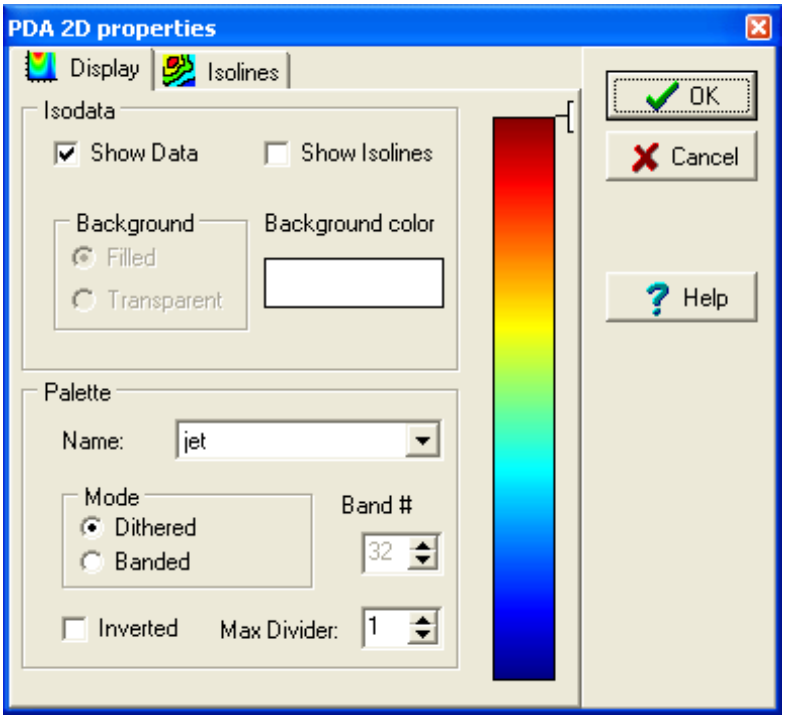
Use the following icons or scrolling list to:

	Modify the isocontour view properties, see below.
	Select which response you want to display. Four choices are proposed: Response, Threshold up, Threshold down and Threshold between to display respectively all the absorbance levels with the same importance or emphasize certain ones. All absorbances inferior to the lower limit or superior to the upper limit will have the same color.
	Maximize the isocontour map view to display it in the full area dedicated to PDA views
	Minimize the isocontour map view size and return in the preceding view mode.

Click on the button to change the isocontour view properties.

Two tabs allow you to modify the acquisition displaying properties: Display, Isolines.

Display



In this tab, customize the isocontour view display.

Check or uncheck **show data** to display or hide the acquisition points when *show isolines* is checked. If **show data** is unchecked, only the isolines are displayed.

Check or uncheck **show isolines** to display or hide the isolines.

If *show data* is unchecked, you can change the background color. Click on the mouse left button in the **background color** field and choose a color in the appearing screen. It is also possible to make the background transparent and display the scale grid (select **transparent** in the background field).

In the **palette** field customize the color scale of the view. The resulting scale is displayed on the screen right column. Change the **name** to modify the absorbance-color correspondence. It is also possible to invert the scale (check **inverted**) or to emphasize certain absorbances by changing the value of the **Max Divider** (see below for more explanation). The coloration can also be **dithered** or **banded**. In this case you can choose the number of bands.

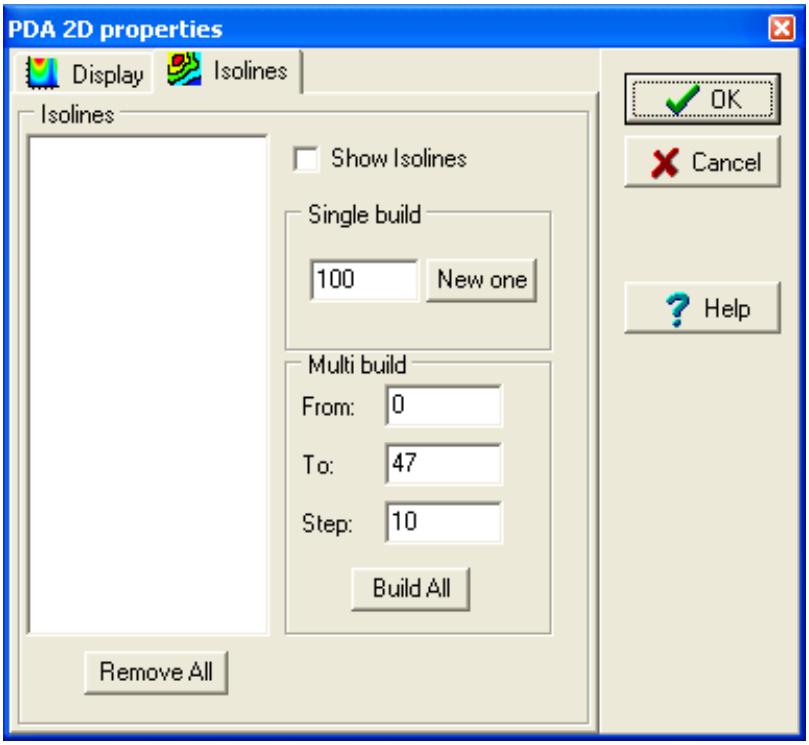
What does the Max Divider Parameter mean?

The Max Divider Parameter will define the limit of color levels displaying the isocontour view.

The inferior limit of the color level 7 (level for the largest absorbance values) will be divided by the max divider parameter. As a consequence, if Max Divider is superior to one, this level range will be larger and will contain more data points: above a certain value of absorbance, the data will be displayed with the same color (the color chosen for level 7).

The other color level ranges (1 to 6) will be narrowest. As a consequence, the small variations will be emphasized on the isocontour view.

In conclusion, if you want to emphasize the small variations of the isocontour display, choose a large value.



In the **Isoline** tab, check **Show Isolines** if you want to display isolines.

Choose the absorbance line you want to display in the single build field and then click on **New one**.

To automatically build the isolines, choose in the **Multi build** field, the minimum absorbance (From: field), the maximum absorbance (To: field) and the Step. Then click on *Build All.*

After choosing the isolines, their absorbance values are given in the Isolines field. You can uncheck the isolines you do not want to display.

The Chromatogram View

Colors of chromatograms are set by default and cannot be customized.

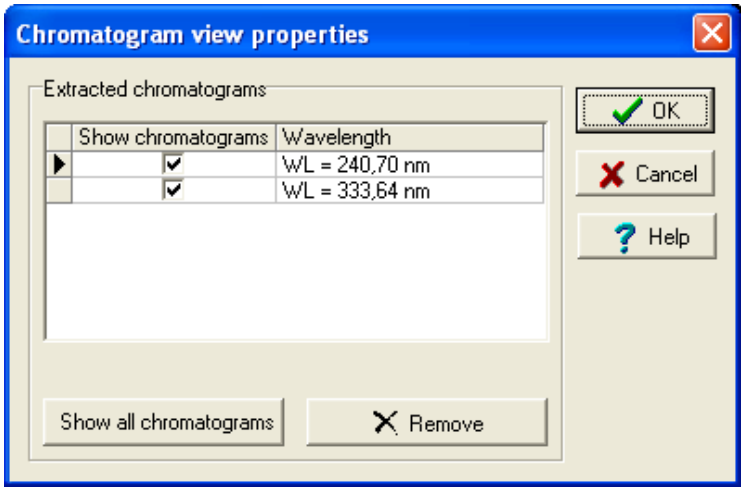
Use the following icons to:

Modify the chromatogram view properties, see below.

Maximize the chromatogram to display it in the full area dedicated to PDA views

Minimize the chromatogram view size and return in the preceding view mode.

Click on the button to change the chromatogram view properties. The following screen appears:



Choose which chromatogram(s) you want to display by checking the corresponding box in the *Show chromatograms* column.

Press the *Remove* button to delete chromatogram(s) you do not want to display.

Press the *Show all* button to display all the listed chromatograms.

The Spectrum View

Colors of spectra are set by default and cannot be customized.

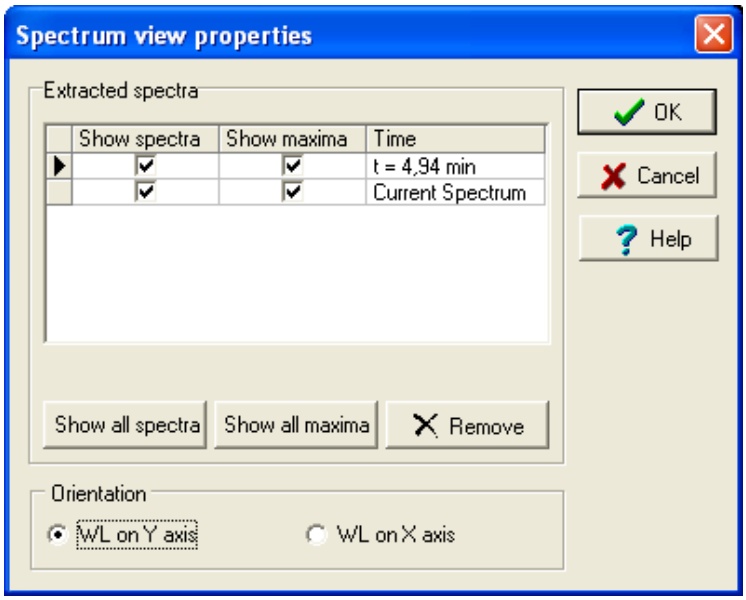
Use the following icons to:

Modify the spectrum view properties, see below.

Maximize the spectrum to display it in the full area dedicated to PDA views

Minimize the spectrum view size and return in the preceding view mode.

Click on the button to change the spectrum view properties. The following screen appears:



Choose which spectrum(a) you want to display by checking the corresponding box in the *Show spectra* column.

Choose which spectrum maximum(a) you want to display by checking the corresponding box in the *Show maxima* column. You can also choose to hide/show given maximum(a) by clicking on their box directly in the spectrum view.

Press the *Show all spectra* button to display all the listed spectra.

Press the *Show all maxima* button to display the maxima of all the selected spectra.

Press the *Remove* button to delete spectrum(a) you do not want to display.

Select the *WL on Y axis* or *WL on X axis option* to display the spectrum vertically or horizontally.

The 3D View

Use the following icons or scrolling list to:

Modify the 3D view properties, see below.

Response — Select which response you want to display. Four choices are proposed: Response, Threshold up, Threshold down and Threshold between to display respectively all the absorbance levels with the same importance or emphasize certain ones. All absorbances inferior to the lower limit or superior to the upper limit will have the same color.

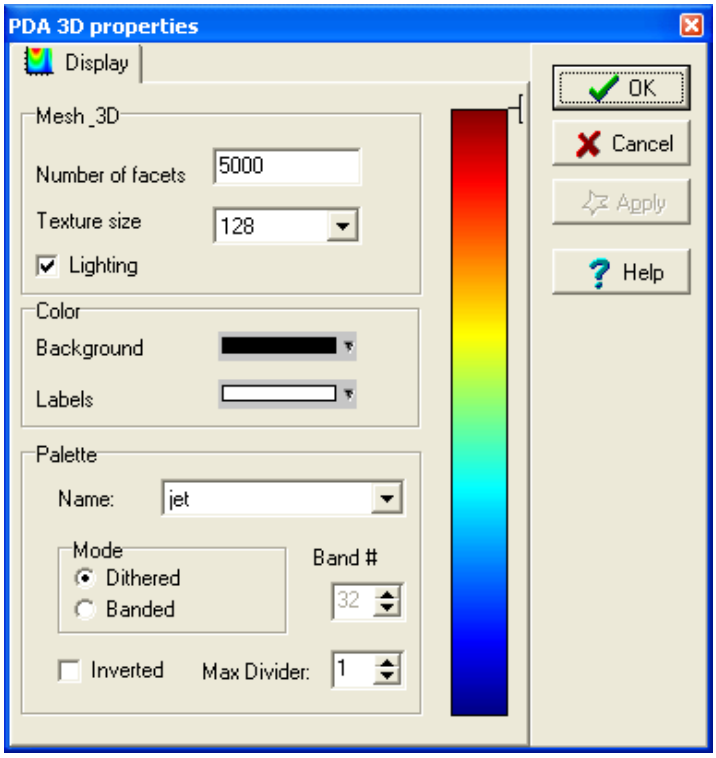
Create the 3D view. By default, the 3D view is empty.

Synchronize the zoom between the views.

Maximize the 3D view to display it in the full area dedicated to PDA views

Minimize the 3D view size and return in the preceding view mode.

Click on the button to change the 3D view properties. The following screen appears:



You can modify the appearance of the 3D object:

The number of facets and the texture size can be modified in the Mesh-3D field.

The color of the background and the labels can be chosen in the color field.

In the palette field customize the color scale of the view. The resulting scale is displayed on the screen right column. Change the name to modify the absorbance-color correspondence. It is also possible to invert the scale (check **inverted**) or to emphasize certain absorbances

by changing the value of the **Max Divider** (see below for more explanation). The coloration can also be dithered or banded. In this case you can choose the number of bands.

What does the Max Divider Parameter mean?

The Max Divider Parameter will define the limit of color levels displaying the 3D view.

The inferior limit of the color level 7 (level for the largest absorbance values) will be divided by the max divider parameter. As a consequence, if Max Divider is superior to one, this level range will be larger and will contain more data points: above a certain value of absorbance, the data will be displayed with the same color (the color chosen for level 7).

The other color level ranges (1 to 6) will be narrowest. As a consequence, the small variations will be emphasized on the 3D view.

In conclusion, if you want to emphasize the small variations of the 3D display, choose a large value.

Synchronization of the Views

In the '4views' or '3views' modes, you can apply the same zoom on all the views or work independently in each one. This can be done using the pop-up menus of each view.

Choose *Synchronized mode* to always synchronize the zoom in all the views.

Choose *Cursor mode* so that when you move the cursor in one of the views using the mouse, the corresponding chromatogram and/or spectrum is displayed in the corresponding view(s).

Unselect *Synchronized mode* in order to work independently in each views. It is nevertheless possible to synchronize punctually the chromatogram and/or spectrum afterwards with isocontour views using the following functions:

- *Synchronize views* from the isocontour popup menu: apply the same zoom on all the views.
- *Synchronize chromatogram with isocontour view* from the chromatogram popup menu: apply the isocontour view zoom on the chromatogram only. The spectrum(a) stay(s) unchanged.
- *Synchronize with isocontour view* from the spectrum popup menu: apply the isocontour view zoom on the spectrum only. The chromatogram(s) stay(s) unchanged.

Note: How to zoom: In each view, zoom in or out by clicking and dragging the left mouse button (from top-left to bottom-right to zoom in or bottom-right to top left to zoom out) or use the *Zoom in* or *Zoom Out* function of the pop-up menus.

Displaying Chromatograms and Spectra

Getting Chromatograms and Spectra

Many ways exist in CompassCDS Data System to fix chromatograms or spectra:

- First, you can fix the chromatogram or the spectrum at the current mouse cursor by clicking respectively on *get chromatogram* or *get spectrum* in each view pop-up menu.
- You can also fix a chromatogram or a spectrum by keyboard typing the wavelength value or the time. Click on *Get chromatogram at WL* in the chromatogram popup menu or *Get spectrum at time* in the spectrum popup menus of each view.
- At least, you can fix spectra by enabling the *Spectrum selection* function in the chromatogram popup menu of the 'spectral extraction' mode. Select the area where you want to display the spectrum(a). Now, move the cursor over the chromatogram and double click once the cursor is correctly positioned to fix the spectrum. Several spectra can be fixed successively still *Spectrum selection* is enabled.

Once chromatograms and spectra have been fixed you can display or hide them in each view. Press the button and in the appearing screen respectively check or uncheck the chromatogram(s) or spectrum(a). Press the *remove* button to delete chromatogram(s) or spectrum(a) you do not want to display. Press the *display all* button to display all the chromatogram or spectra listed above.

The maximum absorbance chromatogram can also be displayed. Choose *Show max absorbance chromatogram* in the chromatogram pop-up menus of the '4views', '3views' or 'spectral extraction' views. The maximum absorbance wavelength is given at each chromatogram points when you move the mouse cursor over the chromatogram.

Note: The maximum absorbance chromatogram is a virtual chromatogram which shows all components at their maximum absorbance. Within the selected wavelength range (full range or user defined range), CompassCDS takes the highest absorbance value from the spectrum and uses it as a chromatogram data point to generate the 2D chromatogram data field (spectrum time as x-value, highest absorbance value as y value). This chromatogram is very useful for quantitative calculation. The wavelength at which the maximum absorbance has been found is displayed in the top of the chromatogram when you left click on the chromatogram.

Normalization of the Spectra Views

Use the normalization function to apply a specific scale on the spectra absorbance axis. This can be done from the spectrum popup menus of the '4views', '3views' or 'spectral extraction' views.

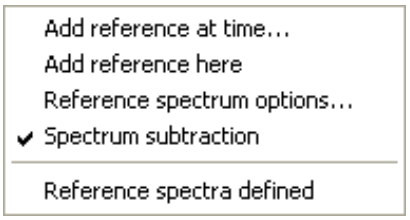
Click on *Normalized View,* a submenu is displayed with two choices (**auto** and **at WL**). Depending on the chosen option, the spectra are zoomed in such a way that the signal corresponding to the maximum absorbance (auto mode) or the absorbance at the specified wavelength of all the displayed spectra (**at WL** mode) represents 5 to 95% of the absorbance axis.

Spectrum Subtraction

Use the Spectrum subtraction function to subtract one spectrum (got at a specific time) or the spectrum interpolated between two reference spectra to all other spectra. This can be

done from the chromatogram popup menus of the '4views', '3views' or 'spectral extraction' views.

Click on the *Spectrum subtraction* option to select a spectrum to subtract. The following screen appears:



Choose **Add reference at Time** and enter manually a time where a reference spectrum will be defined in the appearing screen.

Click on **Add reference here** to define a reference spectrum at the cursor time.

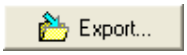
Choose **reference spectrum options** to remove some reference spectra.

Check **Spectrum subtraction** to activate or not the subtraction. A spectrum is interpolated between two consecutive reference spectra and subtracted to all spectra of the corresponding interval. The results of the subtraction are given in the spectrum view.

When reference spectra have been defined, "Reference spectra defined" appears in the bottom part of that popup menu. Else, "No reference spectrum defined" appears.

The ASCII Export of Spectra

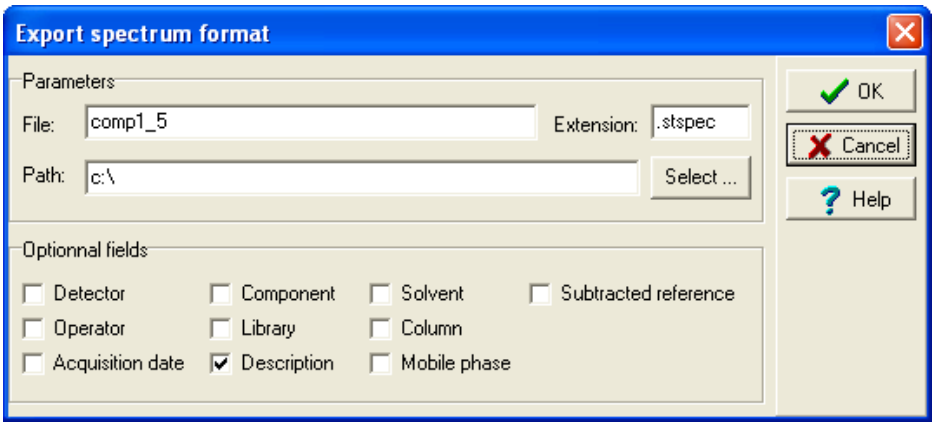
Spectral Data can be exported with the ASCII format.

To perform this export, click on the 

button in the Spectral Library Editor window to export a spectrum stored in a library.

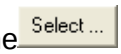
Otherwise, from the spectrum view of the 3-view, 4-view or spectral extraction mode, use the pop-up menu and the 'Spectrum processing' then 'Export spectrum' option.

The following screen appears:



This window is divided into 2 parts, the 'Parameters' of the export and some 'Optional Fields' if a reference spectrum from a library is to be exported and 3 parts if a spectrum from a spectrum view is going to be exported.

In the 'PARAMETERS' part, you can choose the name of the spectrum listed in the library you want to export and the path where you want to export this file. Be sure you have writing rights on the export directory.

You can type in the export path or use the 

button to use a browser to select it.

In the 'OPTIONAL FIELDS', you can check boxes corresponding to the information you want to export:

- Detector: if this box is not checked, the name of the detector the spectrum was recorded with, will not be exported.
- Operator: if this box is not checked, the name of the operator who ran the acquisition of the spectrum will not be exported.
- Acquisition date: if this box is not checked, the acquisition date of the spectrum will not be exported
- Component: if this box is not checked, the component information corresponding to the spectrum will not be exported
- Library: if this box is not checked, the name of the library the spectrum was stored in will not be exported.
- Description: if this box is not checked, the description of the not be exported
- Solvent: if this box is not checked, the solvent information (if any) will not be exported
- Column: if this box is not checked, the column information (if any) will not be exported
- Mobile Phase: if this box is not checked, the mobile phase information (if any) will not be exported
- Subtracted reference: if this box is not checked, the name of the reference spectrum subtracted to the exported spectrum will not be exported.

Once the export parameters have been set, click on the *OK* button. The following message appears then, informing the user that the export is successful:

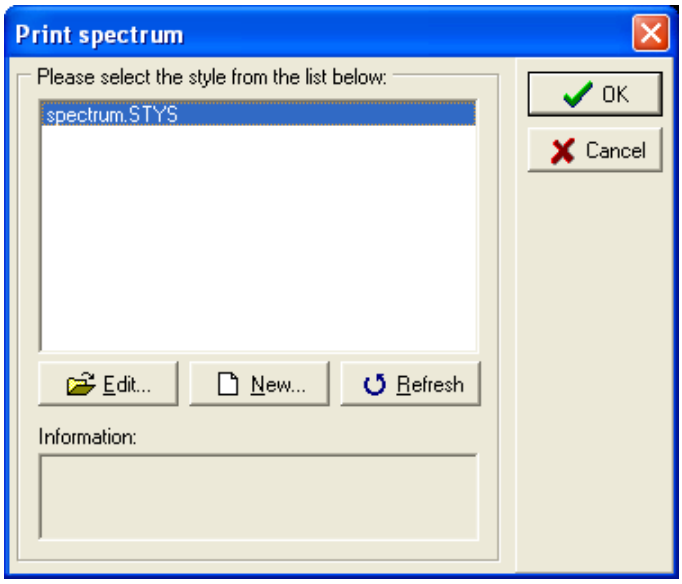
Note: See page 44 for more details about the CompassCDS ASCII format.

The Spectra Report Editor

CompassCDS Spectra Report Editor enables you to generate, customize, and print spectral reports from the CompassCDS.

This feature is accessible from the spectra view (in the 4-view, 3-view and spectral extraction modes), it is possible to preview or print a spectra report by clicking either on the or the buttons.

The following screen is displayed:



When the user selects a defined report in the list, he can edit it by pressing the *Edit* button; create a new one by pressing the *New* button to customize the report, or refresh the list by pressing the *Refresh* button.

The CompassCDS Spectrum Report editor is open where user can choose to add standard objects and Spectra Report Object.

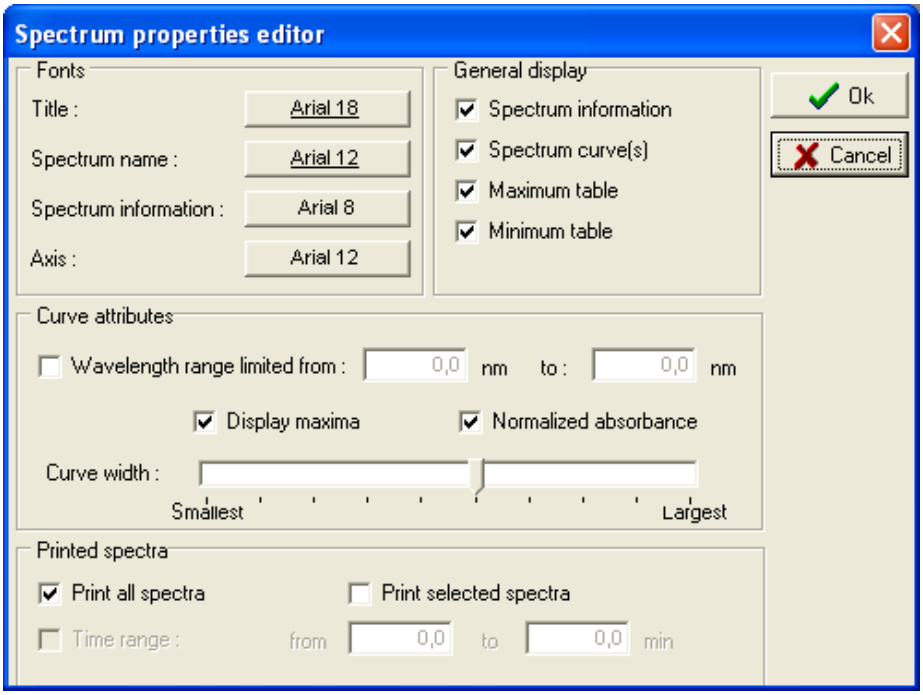
Standard Objects

Concerning standard object such as text, table or graphic objects, please refer to the CompassCDS report Editor Manual.

Spectra Report Object

This object enables you to create and to insert a spectra report. You can define the spectra to print, font style, and general display parameters.

In the pop-up menu select PROPERTIES to configure the object. The following window appears:



In the '*Fonts*' part, you can choose the font of the different items that are to be printed such as the title or the spectra names police size.

In the *General Display* part, you can check the boxes corresponding to:

- Library header: if this box is not checked, the name of the printed library, the number of reference spectra listed, the creation date and description will not be displayed on the report.
- Spectrum table: if this box is not checked, information on the spectra present in the library (such as the compound names, the retention times, and their description and acquisition window) will not be printed.
- Spectrum information: if this box is not checked, information on the printed spectra (such as the name of the chromatogram file it was extracted from, the detector name of the system, the experimental conditions (solvent, column) or the acquisition date) will not be printed in the report.
- Spectrum curve: if this box is not checked, no spectrum curve will be printed in the report
- Maximum table: if this box is unchecked, the wavelength at the maximal absorbance and the value of the maximal absorbance will not be displayed on the report.
- Minimum table: if this box is unchecked, the wavelength at the minimal absorbance and the value of the minimal absorbance will not be displayed on the report.

In the *'Curve Attribute'* part, you can customize the curve by setting the wavelength range that will be printed, if maxima will be displayed, if the absorbance will be normalized or not and the width of the drawing

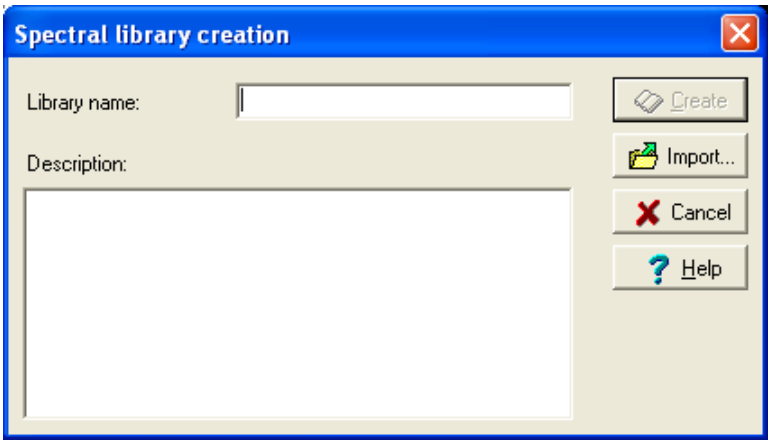
Finally, you can choose which spectra are to be printed: all of them, any consecutive ones listed in the library, those corresponding to a defined time range, or only the ones named in this screen.

PDA Data Processing

The Spectral Library Editor

Creating a New Library

In the **File** menu, click on the *New / New spectral library* option. The following screen appears:

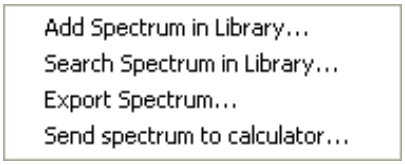


Enter the name of the new library and then click on the *Create* button. User can also add a description. This description will then be displayed when selecting this library in the other screens.

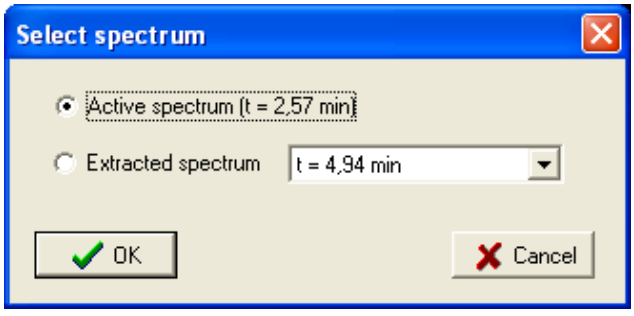
Click on the *Import* button to import in one step several ASCII spectra in a Library. You can give it a name or recover the name of an old library (exported from the Star Software).

How to Fill the Spectral Library

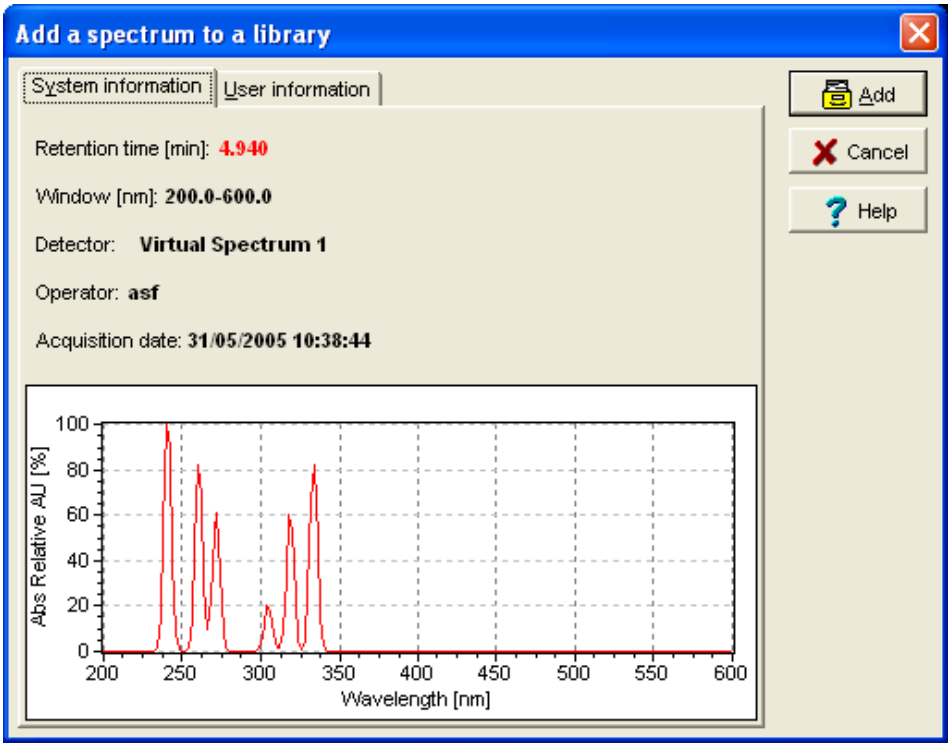
You can add specific spectrum in the library using the pop-up menus of different areas: the spectrum field (from the '4 views' '3 views' or 'spectral extraction' screens) or the isocontour field (from the '4views' or '3 views' screens). Right-click and choose the **Spectrum processing** menu and the following sub menu appears:



Now choose **Add Spectrum to Library** and select which spectrum you want to add in the following sub menu:



The following screen appears:

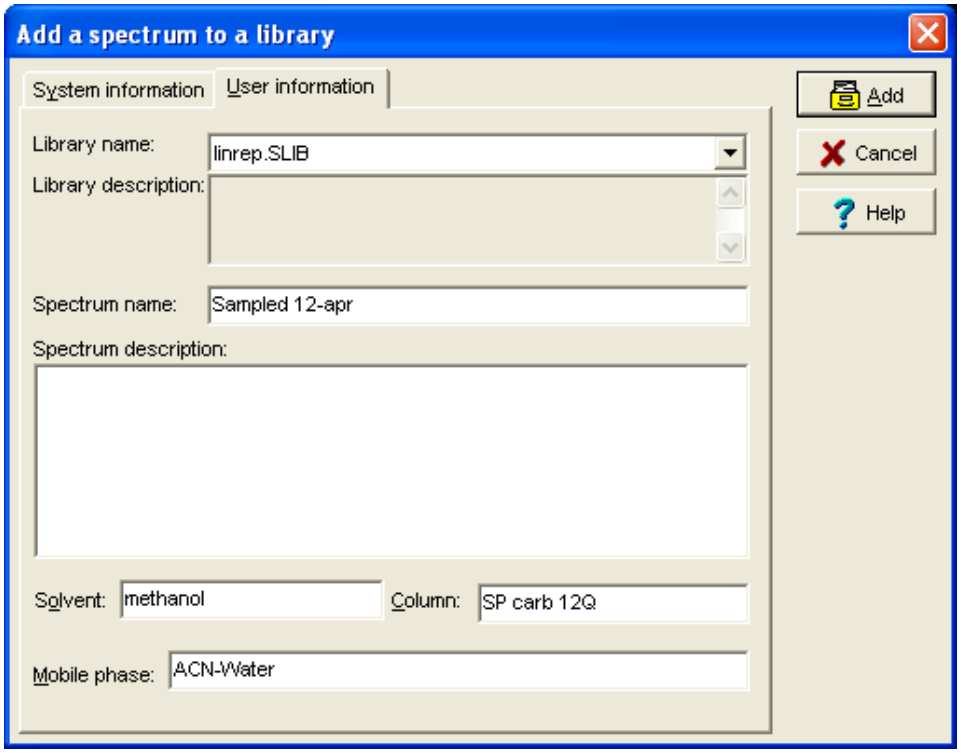


The following is displayed on this page:

- The retention time from which the spectrum is extracted.
- The acquisition wavelength range (Window field)
- The detector type
- The operator name
- The acquisition date

The added spectrum is displayed in this page. Click on the *Add* button to add the selected spectrum. Click on *Cancel* to close the library without adding the selected spectrum.

Click on the ***User Information*** tab. The following screen appears:

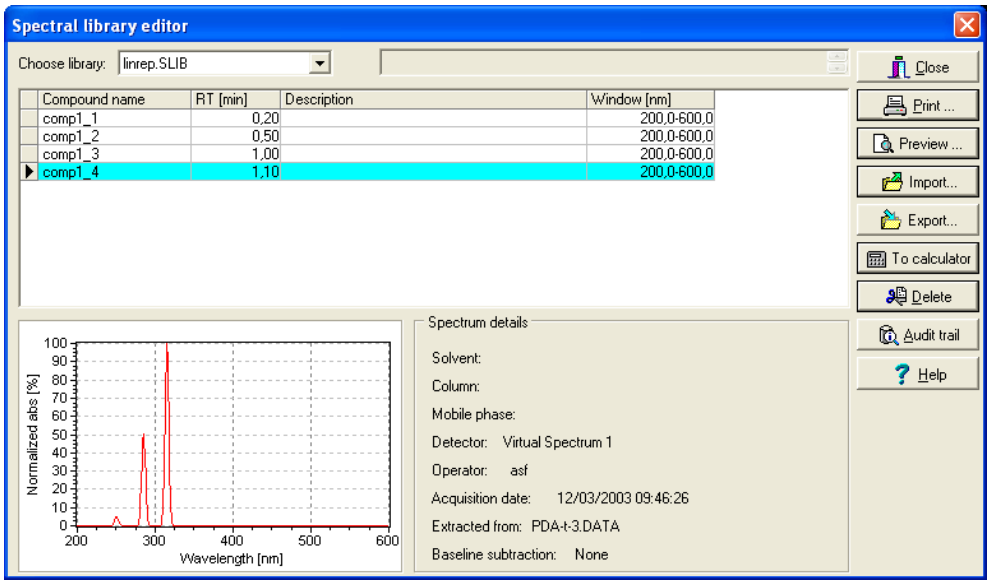


In the **Library name** field, choose a library using the scrolling list. The description entered at library creation is automatically displayed in the Library description field.

Enter the spectrum name and optionally, any description concerning the extracted spectrum. Additional information can be entered such as **Solvent** name, **Column** type, **Mobile Phase.**

Open a Library

In the **File** menu, click on *Open / Open spectral library*. The following screen appears:



In the **Choose library** field, choose a library using the scrolling list. If spectra are stored in this library:

- the name of the spectrum appears in the compound name
- the acquisition wavelength range is specified in the Window column

When you choose one spectrum, the spectrum curve appears in the left part of the screen and the different fields (Solvent, Column, Mobile Phase, Detector, Operator, Acquisition date, Extracted from and baseline subtraction) are filled. This information is given by the user when a spectrum is added to the library, see page 41.

To delete a spectrum, click on its name and then on the *Delete* button.

The ASCII Import of Spectra

Spectra can be imported with the ASCII format.

From the Spectral Library, click on the

button.

An 'Open' box opens and the user is asked to select in the Windows browser the file he wants to import.

The newly imported spectrum is then displayed with all the information contained in it: experimental conditions, operator, acquisition date, etc.

In the description field, 'imported' is displayed to warn the user this spectrum has been imported

The CompassCDS ASCII format of spectra

Mandatory Data

Chromato Extracted From:	Name of the chromato file, the spectrum had been extracted.
RT:	Time of the extracted spectrum.
Number of Points:	Number of points in the spectrum
x0 : y0 :	Points List: x ⇔ Time, y ⇔ Absorbance.
.	
.	
xn : yn :	

Additional Data

Component Name:	Name of the component spectrum.
Library Name:	Name of the library the spectrum comes from.
Acquisition Date:	Date of the chromato acquisition / spectrum extraction (“MM/DD/YY HH:MM:SS”)
Description:	Spectrum description.

Detector:	Name of the detector used for acquiring this spectrum
Operator:	Name of the operator who performed this spectrum acquisition
Solvent:	Name of the solvent used for acquiring this spectrum
Column:	Name of the column used for acquiring this spectrum
Mobile Phase:	Name of the mobile phase used for acquiring this spectrum.
Ref Subtracted; RST:	Has a reference spectrum be subtracted to the extracted one? And Time of the reference spectrum if any.

Other Conventions

Fields Separator:	**;**
Decimal separator:	**.**
File extension:	

Suggestions:

Another ASCII file could be defined to describe the library grouping different spectra, with fields like:

- Library Name
- Library Description
- Library History

The Spectral Library Report Editor

CompassCDS Spectral Report Editor enables you to generate, customize, and print spectral reports from the CompassCDS.

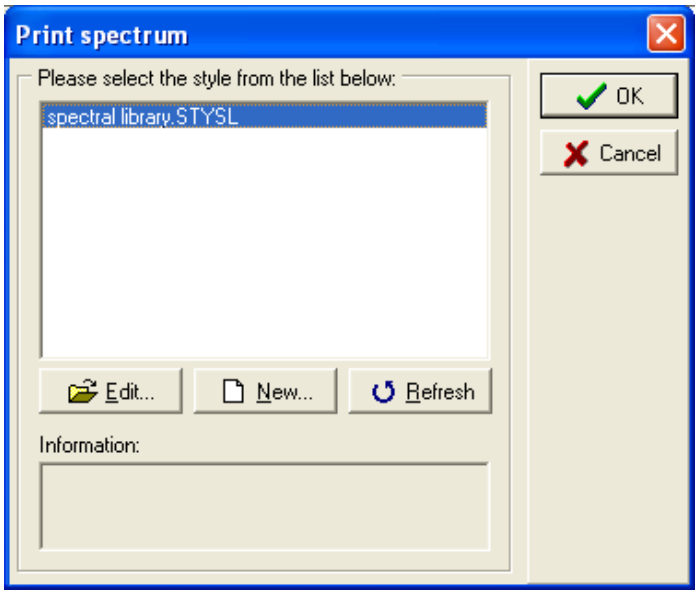
In CompassCDS Report Editor, all the objects can be placed and sized inside pages as they will be printed.

The CompassCDS Report Editor graphical environment is composed of a main menu, toolbars, a browser, a working space, and a status bar.

To print or print preview a Spectral Library, open it then click on the

or

icon. The following screen is displayed:



When the user selects a defined report in the list, he can edit it by pressing the *Edit* button, create a new one by pressing the *New* button to customize the report, or refresh the list by pressing the *Refresh* button.

The CompassCDS Spectrum Report editor is open where user can choose to add standard objects and Spectra Report Object.

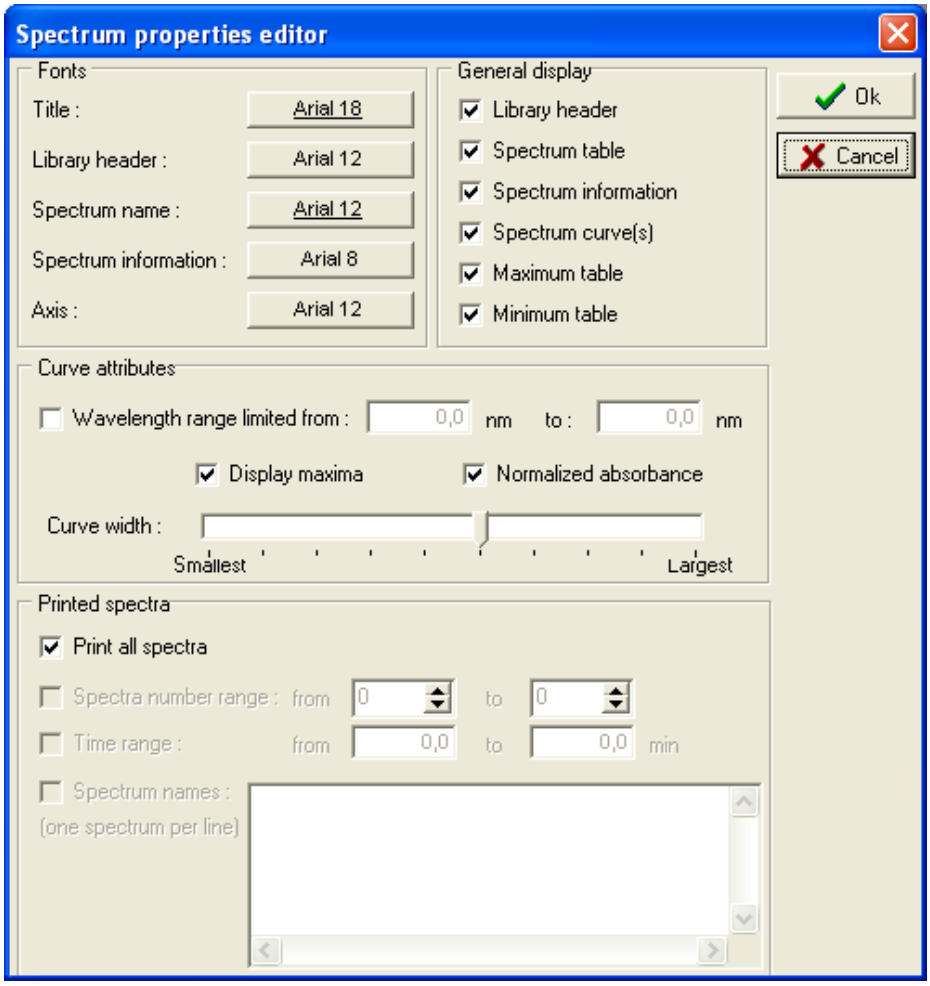
Standard Objects

Concerning standard object such as text, table or graphic objects, please refer to the CompassCDS report Editor Manual.

Spectra Report Object

This object enables you to create and to insert a spectra report. You can define the spectra to print, font style, and general display parameters.

In the pop-up menu select PROPERTIES to configure the object. The following window appears:



In the '*Fonts*' part, you can choose the font of the different items that are to be printed such as the title or the spectra names police size.

In the *General Display* part, you can check the boxes corresponding to:

- Library header: if this box is not checked, the name of the printed library, the number of reference spectra listed, the creation date and description will not be displayed on the report.
- Spectrum table: if this box is not checked, information on the spectra present in the library (such as the compound names, the retention times, the description and acquisition window) will not be printed.
- Spectrum information: if this box is not checked, information on the printed spectra (such as the name of the chromatogram file it was extracted from, the detector name of the system, the experimental conditions (solvent, column) or the acquisition date) will not be printed in the report.
- Spectrum curve: if this box is not checked, no spectrum curve will be printed in the report
- Maximum table: if this box is unchecked, the wavelength at the maximal absorbance and the value of the maximal absorbance will not be displayed on the report.

- Minimum table: if this box is unchecked, the wavelength at the minimal absorbance and the value of the minimal absorbance will not be displayed on the report.

In the *'Curve Attribute'* part, you can customize the curve by setting the wavelength range that will be printed, if maxima will be displayed, if the absorbance will be normalized or not and the width of the drawing

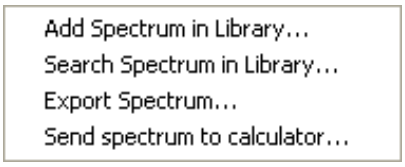
You can finally choose which spectra are to be printed: all of them, any consecutive ones listed in the library, those corresponding to a defined time range, or only the ones named in this screen.

The Library Search

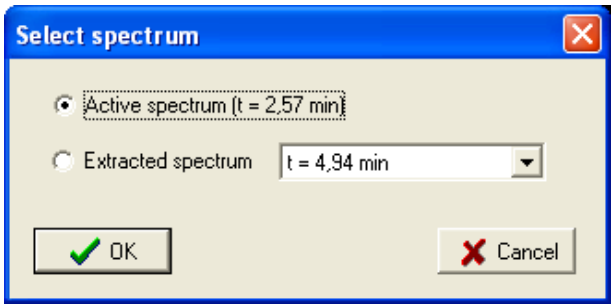
This option allows the operators to identify an unknown spectrum by searching the similar spectrum in the library. This option can be used manually or automatically. The manual search is useful to define the appropriate search parameters or to quickly identify some unknown. The manual search can also find the library spectrum to be used as reference for purity calculation. A link exists which allows to define this reference spectrum directly from the manual search screen.

The Manual Search

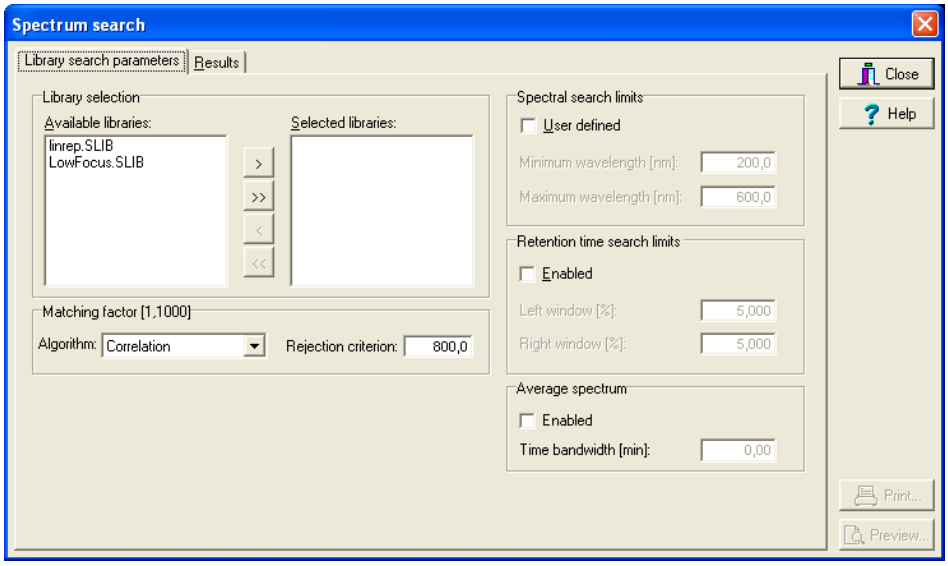
The manual search can be performed using the pop-up menus of different views: spectrum pop-up menus (from the '4 views', '3 views' or 'spectral extraction' views) or isocontour pop-up menu (from the '4views' or '3 views' views). Right-click and choose the **Spectrum processing** menu and the following sub menu appears:



Now choose **Search spectrum in Library** and select which spectrum you want to compare in the following screen:

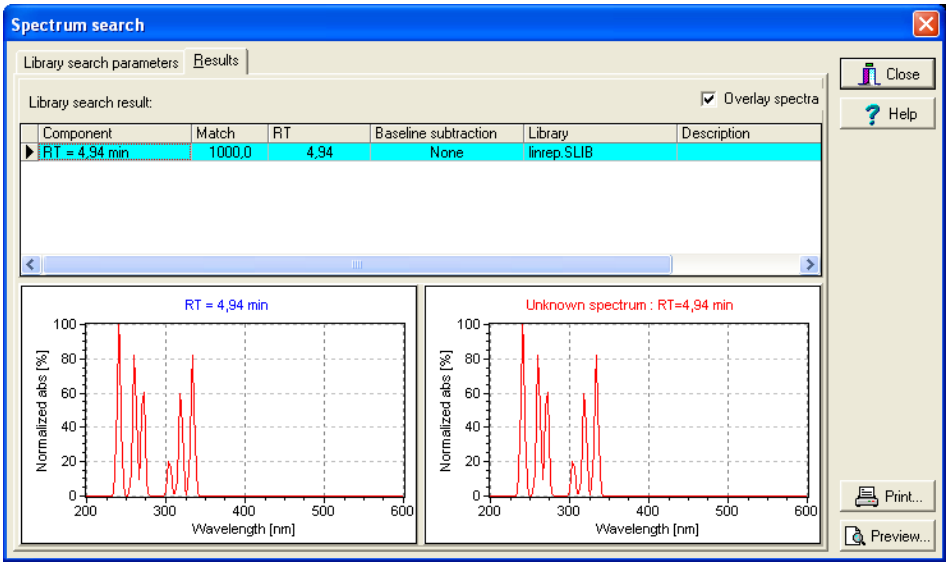


The Spectrum Search screen consists of two tabs. Choose the **Parameters** one. The following screen appears:



Select the parameters used to search the spectrum in the libraries. The parameters definition is given beginning on page 52.

Click on the *Results* tab, the following screen appears:



The correlations are calculated and the candidate spectra are displayed in the spectrum table. For an unknown spectrum, the table gives the library spectrum name, its retention time, the matching factor, the library it comes from and the baseline subtracted if any. The components are listed in their matching factor order.

Select a table line to display the corresponding component curve in the field at the bottom of the window. By default, the **overlay spectra** box is unchecked and the spectra are displayed on different windows. The unknown and library spectra can be overlaid. (Check the **overlay spectra** box.)

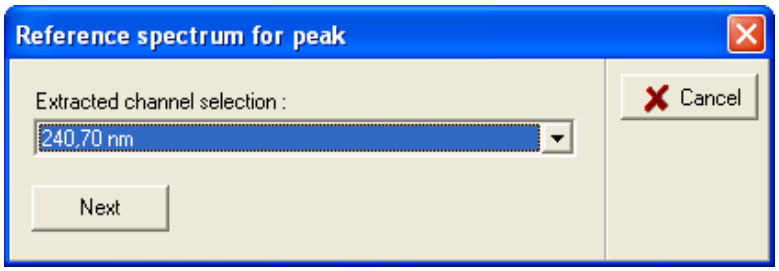
In both cases, the spectra are normalized to 100%.

Note: The results of a manual search cannot be saved

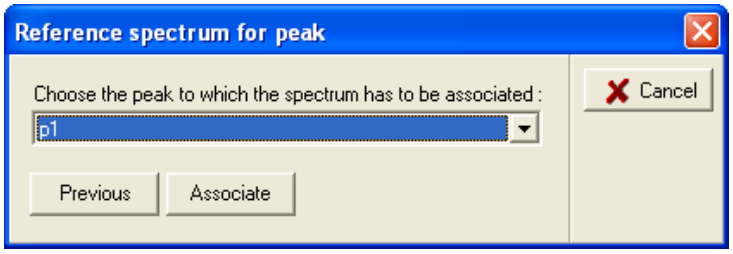
Use the or button to print or print preview the results (see the Manual Library Search Editor section page 50 for details).

Link with the Peak Identification Table of Peak Purity Calculations

It is also possible, from the result tab, to define which library spectrum must be used as reference for purity calculations. Right -click on the spectrum you want to use as reference and click on *Reference spectrum for purity computations.* The following screens appear:



In this screen, select the extracted chromatogram in which the reference spectrum will be associated. Press *next* to display the following screen:



Now, choose the peak to which the spectrum must be associated and press *associate* to associate them.

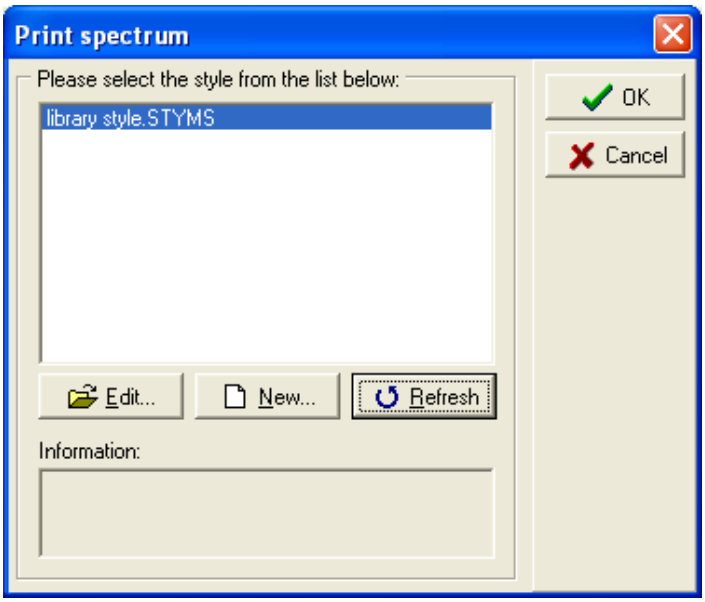
The Manual Library Search Report Editor

CompassCDS Library Search Report Editor enables you to generate, customize, and print manual library search results reports from the CompassCDS.

In CompassCDS Report Editor, all the objects can be placed and sized inside pages as they will be printed.

The CompassCDS Report Editor graphical environment is composed of a main menu, toolbars, a browser, a working space, and a status bar.

To print or pint preview Manual Library Search results, click on the Print ... or Preview ... button. The following screen is displayed:



When the user selects a defined report in the list, he can edit it by pressing the *Edit* button; create a new one by pressing the *New* button to customize the report, or refresh the list by pressing the *Refresh* button.

The CompassCDS Manual Library Search report editor is open where user can choose to add standard objects and Library Search Report Object. Please refer to the CompassCDS report Editor Manual for details.

The Automatic Search

The parameters of the automatic library search are defined in the spectrum method part and are the same for all extracted chromatograms. In the method sub-section of the spectrum section, click on the Edit library search... button to display the screen which allows the user to configure the library parameters (The parameters definition is given beginning on page 43.).

The results for the best matching component can be displayed in the peak results table. Add the Matching component, the matching factor and the matching component library variables (right-click on the peak result table and then choose *report properties*) in the report. Three additional columns appear:

#	Name	Time [Min]	Quantity [% Area]	Area [µV.Min]	Matching Comp	Match.Factor	Matching Comp
1	UNLNOWN	2,48	11,75	17610,5	Benzene	999,794	Demo.SLIB
2	UNKNOWN	4,02	15,78	23641,8	Anthracene	999,996	PAH.SLIB
3	UNKNOWN	5,01	51,59	77304,4	Pyrene	999,955	PAH.SLIB
4	UNKNOWN	7,93	20,89	31297,1	Perylene	999,999	PAH.SLIB
Total			100,00	149853,8			

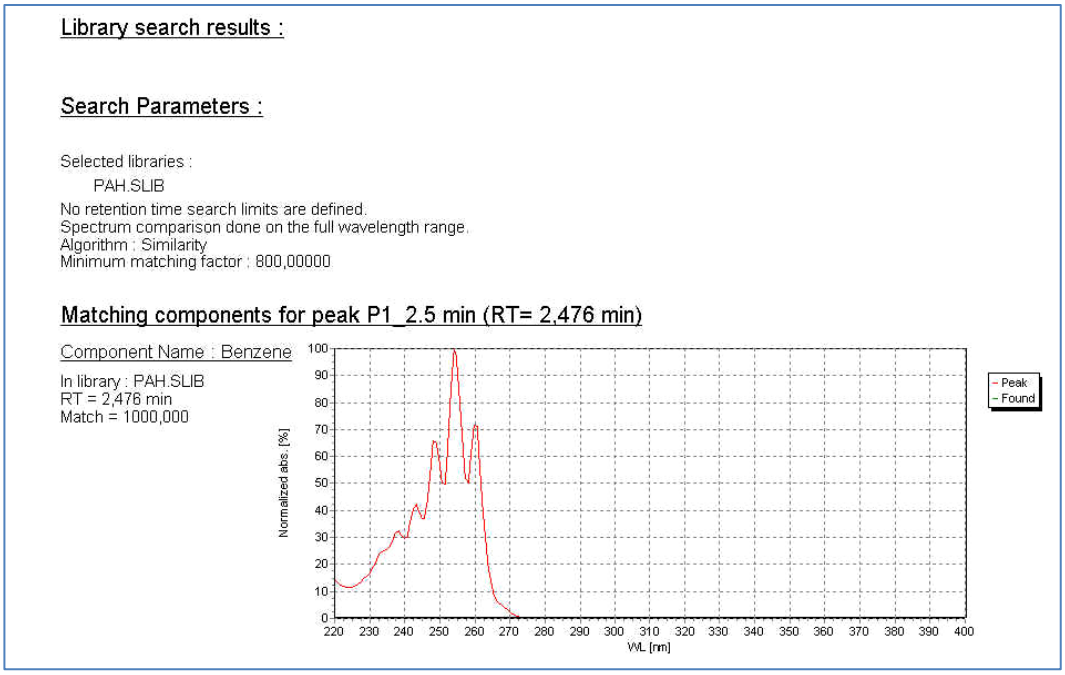
This table can be printed using the peak table object of the CompassCDS Report Editor. Refer to the CompassCDS Report Editor User Guide to see how to build the report style.

The whole library search results can also be displayed or printed via the report editor (CompassCDS Report Editor).

First, define a report style in CompassCDS Report Editor. This style must include a library search object. Refer to the CompassCDS Report Editor User Guide to see how to build the report style.

Then, choose this style in the report sub part of the extracted chromatogram method and click on the button to display a preview of the report or the button to print it.

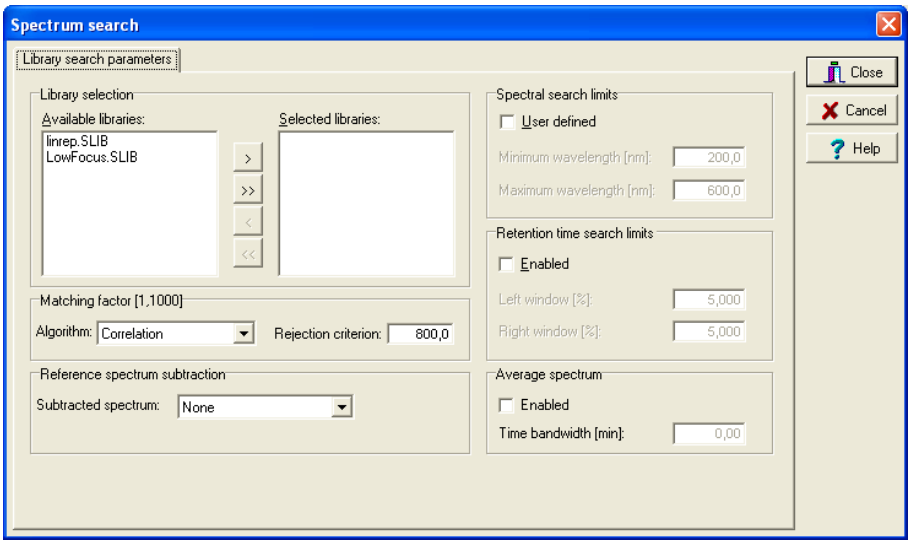
For example, the following image shows a report including the library search results.



According to the CompassCDS Report Editor object properties, the libraries and parameters used for the search can be printed and for an unknown spectrum, the report gives the library spectrum name, its retention time, the matching factor and the library from which it is extracted.

The Search Parameters

Several parameters must be defined to search a spectrum in the library.



Library selection: The unknown spectra can be compared to spectra of several libraries (all spectra of the libraries listed in the *selected libraries* field). To select the libraries choose one or more libraries in the *Available libraries* list and click on the > button to move them in the *Selected libraries* list. Click on >> to select all the available libraries. Click on the < or << button to remove one or all the libraries listed in the *selected libraries* list.

Spectral search limits: By default, the search is realized on the entire spectral range of the unknown (*User defined* box is unchecked) but the user can limited the search in a spectral window (check the *User defined* box and type the chosen value in the appropriate field now enabled). The unknown spectrum will be compared only to library spectra which wavelength ranges are equal or greater than the unknown spectra.

For example:

Unknown spectra **|----------|**

Library spectrum 1 |----------|

Library spectrum 2 |----------|

Library spectrum 3 |----------|

The unknown spectrum will be compared to spectrums 1 and 2, but not to spectrum 3.

Retention time search limits: by default, all library spectra are taken into account for calculation (the *Enabled* box is unchecked). But the user can defined a smaller time window. The unknown spectrum will be compared to spectra which time corresponds to the following formula:

$T_r-\Delta T_g \leq T_{ri} \leq T_r+\Delta T_d$

where T_r is the unknown spectrum retention time

ΔT_g and ΔT_d are the left and right time window

T_{ri} is the library spectrum retention time.

Matching factor: choose the algorithm to use and the Rejection Criterion value, which is the matching factor limit value under which a spectrum from the library is not considered to identify the studied spectrum. Three matching algorithms exist in CompassCDS.

Reference spectrum subtraction: (available in automatic mode only): choose the spectrum to be subtracted. You can select a spectrum to correct the background absorption (this background absorption can be different if the solvent is different from one analysis to another).

- Choose **None** to subtract no spectrum
- Choose **Manual reference** to manually define the time where the spectrum must be extracted. An additional box is added behind the scrolling list where the time can be entered.
- Choose **Peak baseline average** to subtract the spectrum interpolated between peak start and peak stop markers.

Average spectrum: (available in automatic mode only): check *enabled* and define the *time bandwidth* in the enabled field. In that case the spectra compared with the library will be the average of spectra obtained at peak apex over the defined time bandwidth.

The Library Search Results Print

The library search results can be printed as a whole using the report editor (CompassCDS Report Editor).

First, define a report style in the CompassCDS Report Editor. This style must include a library search object. Refer to the CompassCDS Report Editor User Guide to see how to build the report style.

Then, choose this style in the report sub part of the extracted chromatogram method and select 1 in the copy number field. Click on the *preview* button to display the report.

According to the CompassCDS Report Editor object properties, the libraries and parameters used for the search can be printed and for an unknown spectrum, the report gives the library spectrum name, its retention time, the matching factor and the library from which it is extracted.

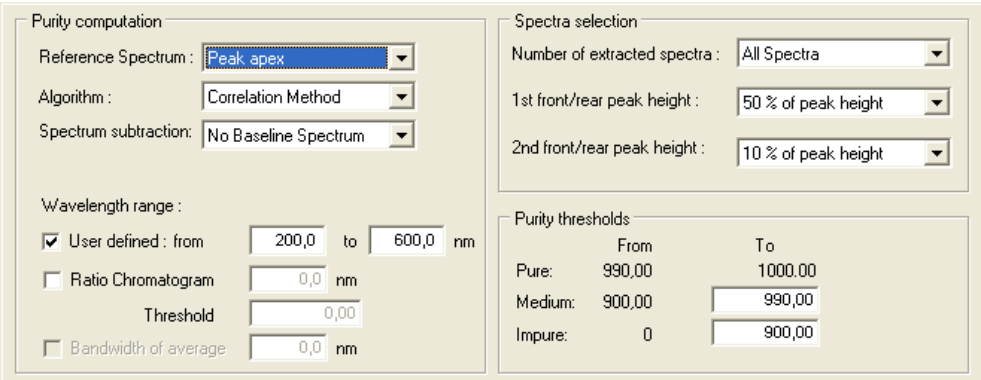
These results can be printed automatically after the acquisition (if the correct report style is defined in the report part of the acquisition method) or manually using the button.

The name of the component that better matches the unknown spectra, the corresponding matching factor and the library it comes from can also be printed. Add in the report a peak table object and associate it a format containing the three variables MATCHCOMPONENT, MATCHFACTOR and MATCHLIBRARY.

The Peak Purity

The Peak Purity Calculation

The peak purity calculation informs the user if a peak is composed of one or several components. In case of co-eluting components, the purity will be very low. The purity test is based on the comparison of spectra stored during the peak elution. Click on the peak purity part of the extracted chromatogram method. The following screen appears:


Purity computation
Reference Spectrum : Peak apex
Algorithm : Correlation Method
Spectrum subtraction: No Baseline Spectrum
Wavelength range :
User defined : from 200,0 to 600,0 nm
Ratio Chromatogram 0,0 nm
Threshold 0,00
Bandwidth of average 0,0 nm
Spectra selection
Number of extracted spectra : All Spectra
1st front/rear peak height : 50 % of peak height
2nd front/rear peak height : 10 % of peak height
Purity thresholds
From To
Pure: 990,00 1000.00
Medium: 900,00 990,00
Impure: 0 900,00

In the **spectra selection field**, you have to choose:
***the number of extracted spectra*.**

Generally, 3 spectra by peak are used to calculate the purity. If necessary, choose apex only, 7 or all spectra.

Now, choose ***the location of these spectra*** in the peak

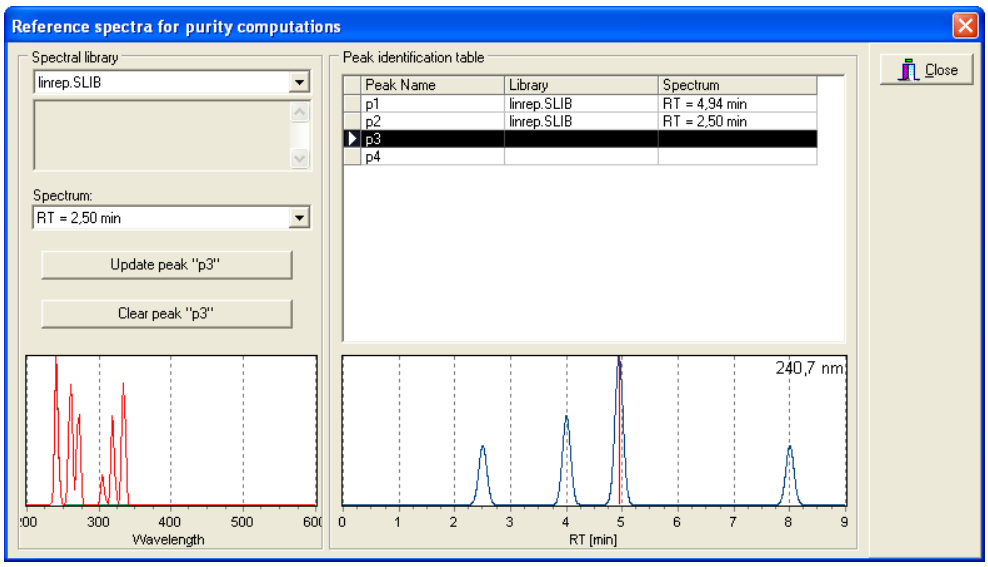
- 50% Peak Height Method: uses the spectrum extracted at 50% of peak height. This measurement is not affected by asymmetry.
- 2 Sigma Method: extracts the spectrum at 60.7% of peak height.
- 3 Sigma Method: extracts the spectrum at 32.4% of peak height.
- 4 Sigma Method: extracts the spectrum at 13.4% of peak height.
- 5 Sigma Method: extracts the spectrum at 4.4% of peak height. The five sigma method is most sensitive to asymmetry because the width is measured at the lowest point.
- 5% of Peak Height Method: extracts the spectrum at 5% of peak height.

The higher the sigma number is, the more accurate the calculation is.

In the *Purity computation* field,

1. Choose the **reference spectrum**: the spectra are compared to the peak spectrum (extract at peak apex) or to library spectra. In this case, first fill the peak identification part and then click in the peak purity part on the Ref button to choose for each spectrum, the library component.

   The following screen appears:



   In the right part of the screen the chromatogram peak names appear. A red cursor indicates in the behind chromatogram which peak is selected. For each peak, choose in the left part of the screen, the library and then the spectrum you want to use to calculate the purity. The unknown and the library spectra are displayed in the behind field respectively in red and green. The library spectrum information is displayed if you let the mouse cursor several seconds on the spectrum name. Click on the *Update peak"i"* button to calculate the purity of peak "i" with the selected library spectrum as reference. The library and spectrum names will be added in the peak identification table. Use the *Clear peak"i"* button to delete a library and a spectrum already present in the peak identification table.

Note: This peak identification table can also be filled from the result tab of the manual library search screen.

2. Choose the **Algorithm** among the 3 available algorithms (refer to The Matching Algorithms of CompassCDS section for detailed definition of the algorithms)

   Whatever the chosen algorithm, the matching factor is always between 0 (impure peak) and 1000 (pure peak).

3. Select the spectrum to be subtracted, you can select a spectrum to correct the background absorption (this background absorption can be different if the solvent is different from one analysis to another).

   Four options are available:

   - Choose **No baseline spectrum** to subtract no spectrum
   - Choose **Manual reference** to manually define the time where the spectrum must be extracted. An additional box is added behind the scrolling list where the time can be entered.
   - Choose **Peak baseline** to subtract the spectra taken at the peak start and stop. The peak start spectrum will be subtracted to spectra of the front part of the peak, e.g.. from the peak start to peak apex and the peak stop spectrum will be subtracted to spectra of the tail part of the peak, e.g., from the peak apex to peak stop. Check the *Use Spectra at Peak Start and Peak Stop* box to extract the spectrum at peak start and peak end markers. Uncheck this box to manually define the peak start and peak end times. These times can be entered in the peak identification table respectively in the RT(FBL) and RT(TBL) columns.
   - Choose **Peak baseline average** to subtract the spectrum interpolated between peak start and peak stop markers.

4. Choose the **wavelength range** for purity computations. If the *user defined* box is unchecked the purity is computed for the full acquisition wavelength range.

5. In the **Purity Thresholds** field, define the range for pure, medium or impure peaks.

   Generally, we consider that:

   A correlation superior to 990 means that peaks are similar

   A correlation between 900 and 990 represents some similarity

   A correlation inferior to 900 indicates that spectra are different.

   You can change these thresholds by modifying the values in the white fields.

6. Check **Bandwidth of average** and defined the bandwidth in the enabled field. In that case the spectra compared will be the average of spectra obtained over the defined time bandwidth.

The Peak Purity Results

The Peak Table

You can view the results of purity processing in the peak table. Select report properties in the peak table pop-up menu and then choose the purity variables you want to display. The following screen shows the results for a search realized on seven spectra compared to the reference:

#	Name	Time [Min]	Pur(Apex)	Pur(FBL)	Pur(FH)	Pur(FL)	Pur(TBL)	Pur(TH)	Pur(TL)
1	P1_2.5 min	2,48	1000,000	0,715	857,412	244,770	63,644	999,526	987,887
2	P2_4 min	4,02	1000,000	0,583	990,529	656,083	249,483	999,635	986,749
3	P3_5 min	5,01	1000,000	1,042	970,834	863,914	161,705	989,220	962,600
4	P4_8 min	7,93	1000,000	74,991	999,299	986,776	423,829	999,786	997,720
Total									

Several variables relative to peak purity can be displayed depending of the chosen mode for purity calculations:

Pur (Apex): this variable gives the peak purity results at the peak apex. This variable is calculated whatever the purity calculation mode.

Pur (FBL): this variable gives the peak purity results at the front baseline. Be careful, this variable is calculated only if "7 spectra" or "all spectra" mode has been chosen for purity calculations.

Pur (FH): this variable gives the peak purity results at the second front peak height. Be careful, this variable is not calculated if "apex only" mode has been chosen for purity calculations. It is calculated in any other modes.

Pur (FL): this variable gives the peak purity results at the first front peak height. Be careful, this variable is calculated only if the "7 spectra" or "all spectra" mode has been chosen for purity calculations.

Pur (RL): this variable gives the peak purity results at the second tail peak height. Be careful, this variable is calculated only if the "7 spectra" or "all spectra" mode has been chosen for purity calculations.

Pur (RH): this variable gives the peak purity results at the first tail peak height. Be careful, this variable is not calculated if "apex only" mode has been chosen for purity calculations. It is calculated in any other modes.

Pur (RBL): this variable gives the peak purity results at the tail baseline. Be careful, this variable is calculated only if the "7 spectra" or "all spectra" mode has been chosen for purity calculations.

AREA_IMPURE: this variable gives the percentage of peak area which is pure. Be careful, this variable is calculated only if "all spectra" mode has been chosen for purity calculations.

AREA_PURE: this variable gives the percentage of peak area which is pure. Be careful, this variable is calculated only if "all spectra" mode has been chosen for purity calculations.

AREA_MEDIUM: this variable gives the percentage of peak area which is pure. Be careful, this variable is calculated only if "all spectra" mode has been chosen for purity calculations.

This table can then be printed. The report style must contain a peak table object which format contains the chosen variables (see CompassCDS Report Editor for more details).

The Ratio Chromatogram

Purity of peaks can also be represented by the Ratio Chromatogram. The absorbance ratio of two wavelengths over time is plotted (it is the ratio chromatogram). If the ratio does not change over time a peak is considered to result from a single compound. Otherwise, the peak is considered to result from several compounds.

To build a ratio chromatogram:

1. In the peak purity subsection of an extracted chromatogram, check the *Ratio Chromatogram* box.
2. Enter the *reference wavelength*. The ratio chromatogram is the ratio of the reference wavelength absorbances by the extracted chromatogram wavelength absorbances.
3. Enter a *threshold*. At each time, if the height of the peak is lower than that threshold, the ratio will be set to zero.

Ratio Chromatograms can be displayed as other Peak Purity results.

The Peak Purity Display (the peak properties)

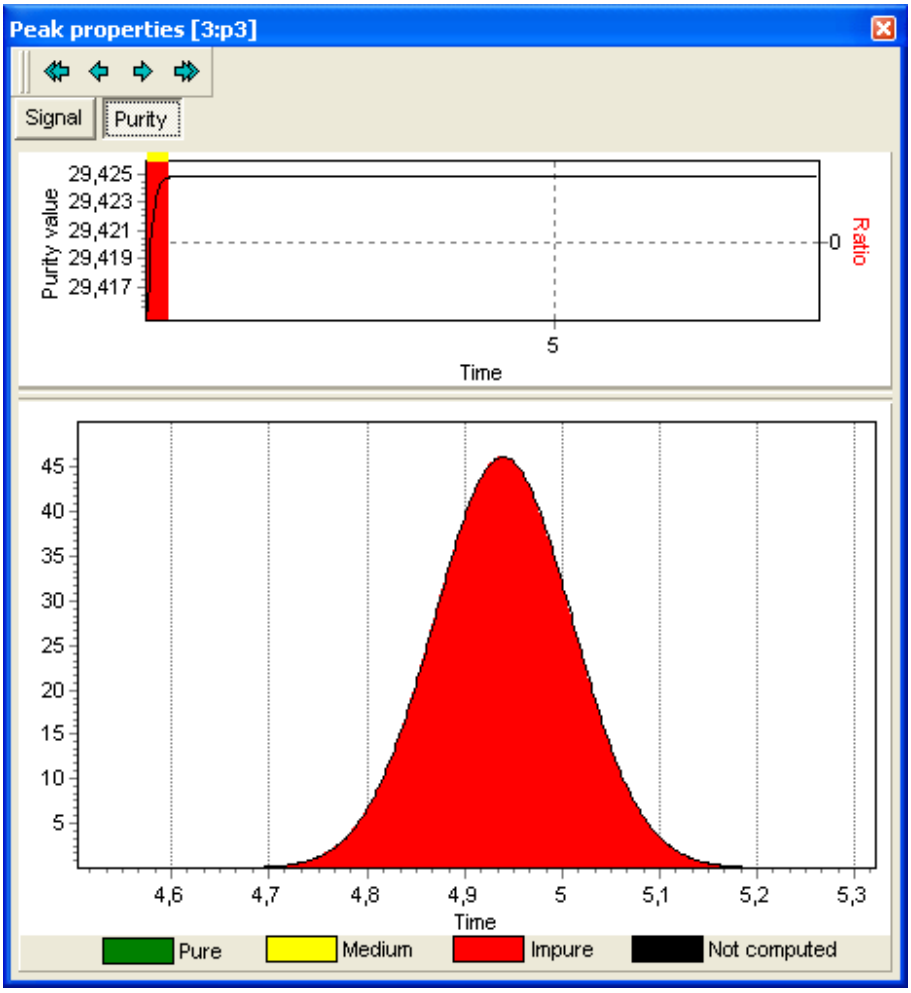
CompassCDS allows visualizing, directly on the peak, area of peak which is pure, impure or medium, a graph relating the peak purity versus the time and the Ratio Chromatogram if any.

When using the method comparing all the spectra (choose *all spectra* in the *number of extracted spectra* scrolling list of the *spectra selection* area), the correlation is calculated at each acquisition point of the peak.

Therefore, each point belongs to one of the three correlation value domains:

- High purity
- Medium purity
- Impurity represented by a lack of correlation

Click on the peak properties icon ( ) and then go in the **Purity** tab. The following screen appears:



On the top part of the screen, the ratio chromatogram and the purity values are displayed. (A graph relating the variation of the purity versus time is also given for each peak. If ratio chromatograms have been defined, the ratio is also displayed here.).

On the bottom part of the screen, the peak purity is displayed in color and the segments of co-elution are instantaneously identified. Click on , , , respectively to display the next peak, the previous peak, the last peak and the first one.

In the case of a comparison not realized on all spectra, each point used for the computation belongs to one of the three correlation value domains. All the other points are displayed in black (Not computed) in the peak properties.

These results can then be printed. The report style must contain a peak purity object (see CompassCDS Report Editor for more details).

The Matching Algorithms of CompassCDS

The correlation method

$$\text{Matching factor} = 1000 \times \frac{\left(\sum_n a_1.a_2 - \frac{\sum_n a_1 \sum_n a_2}{n} \right)^2}{\left(\sum_n a_1^2 - \frac{\left(\sum_n a_1 \right)^2}{n} \right)\left(\sum_n a_2^2 - \frac{\left(\sum_n a_2 \right)^2}{n} \right)}$$

a1 and a2 are the absorbance of the unknown and the reference spectra, measured at the same wavelength.

n: number of point acquired by spectra

Note that Matching Factor = 0 if

$$\sum_n a_1^2 - \frac{\left(\sum_n a_1 \right)^2}{n} = 0 \quad \text{or} \quad \sum_n a_2^2 - \frac{\left(\sum_n a_2 \right)^2}{n} = 0$$

The similarity method

$$\text{Matching Factor} = 1000 \times \frac{\sum_n a_1 a_2}{SQRT\left(\sum_n a_1^{\,2} \times \sum_n a_2^{\,2} \right)}$$

a_1 and a_2 are the absorbance of the unknown and the reference spectra, measured at the same wavelength.

n: number of point acquired by spectra

The dissimilarity method

$$\text{Matching Factor} = 1000 \left(1 - SQRT\left(1 - \frac{\left(\sum_n a_1 a_2 \right)^2}{\sum_n a_1^{\,2} \times \sum_n a_2^{\,2}} \right) \right)$$

a_1 and a_2 are the absorbance of the unknown and the reference spectra, measured at the same wavelength.

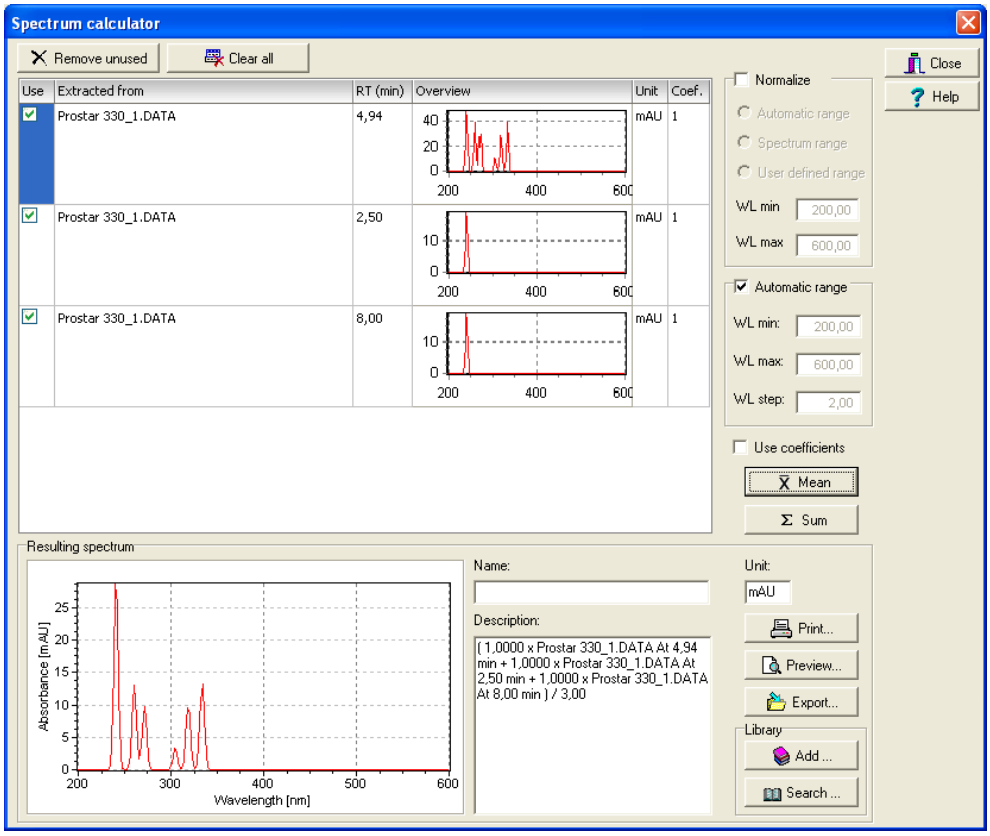
n: number of point acquired by spectra

The Spectrum Calculator

The Spectrum Calculator allows the calculations to be performed (additions and subtractions) on spectra.

To select the spectra you want to perform calculations on, choose 'spectrum processing' then 'send spectrum to calculator' from the pop-up menu in the spectrum window of the 4-view mode or press the "*to calculator*" button from a spectral library.

To open the Spectrum Calculator, click on the button in the CompassCDS toolbar. The following screen appears:



The Spectrum Calculator window is composed of 3 parts: the spectrum list, the parameters settings, and the resulting spectrum part.

The Spectrum List

In the spectrum list, all the selected spectra are displayed with their names, retention times, overviews and the units of the response. All those fields are in read-only mode. In the last column, the coefficient that will be used for the calculations can be set.

Parameters Settings

On the right part of the window, the normalization and computation parameters can be set.

The normalization can be inactive or be set to automatic, spectrum or user defined wavelength range.

- **Automatic range**: all spectra are normalized in the common wavelength range.
- **Spectrum range**: each spectra is normalized in its own wavelength range.
- **User defined range**: all spectra will be normalized in the wavelength range defined by the user.

The computation can be performed on the total wavelength range of the acquisition if the automatic option is checked, or on a user defined range. If the chromatograms have different acquisition wavelength ranges, the calculations will be done on the common range only. The step used for the computation can also be set.

Two actions can be done: 'sum' or 'mean'.

- The **'sum'** option sums all the selected chromatograms, taking each coefficient into account if the "use coefficient" box is checked.
- The **'mean'** action sums all the selected chromatograms, taking each coefficient into account if the "use coefficient" box is checked, and divide it by the sum of coefficient.

Resulting Spectrum

On the bottom of the window, the resulting spectrum is displayed. A name, the unit of the response and a description can be edited.

The resulting spectrum can be previewed and printed (using a report style previously created), exported or added to a spectral library.

A search in library can also be performed to compare this calculated spectrum with reference ones.

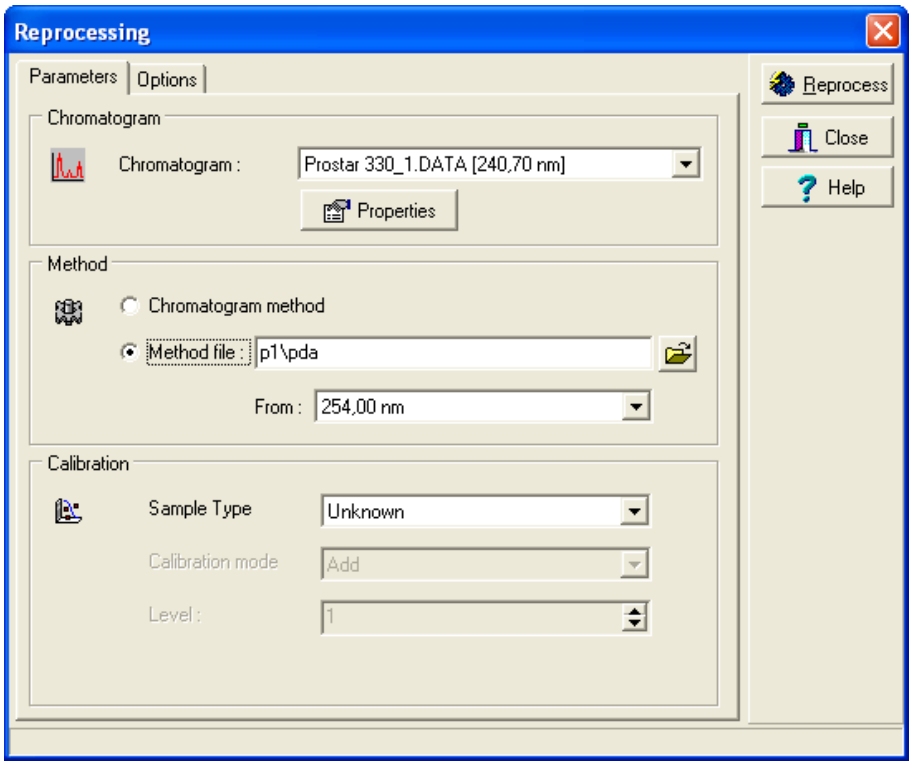
Reprocess the PDA Chromatogram

Once a chromatogram has been acquired, it is possible to reprocess it with another method.

Single Reprocessing

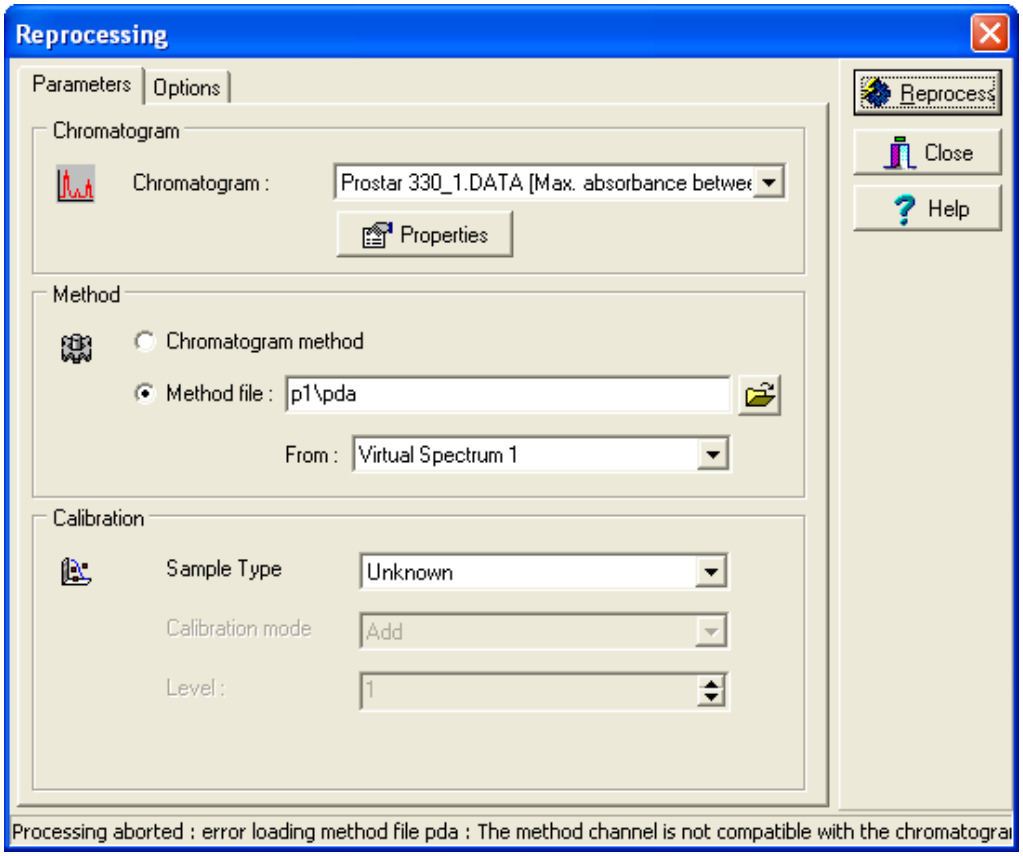
The chromatogram should be opened in CompassCDS to be reprocessed in the single reprocessing window.

Choose the menu *PROCESSING / REPROCESSING / ACQUISITION* to access the reprocessing window (or click on F6, or press the button):



In the Chromatogram zone, select the chromatogram file to reprocess from the list of all the chromatogram names opened in CompassCDS. Choose the right one then choose the method in the corresponding zone. If ‘Chromatogram method’ is chosen, the method inside the chromatogram file is used. If another method has to be used, choose ‘Method file’ to access the open file window to choose the name of the method, and press the open icon ( ).

Be careful to choose the right channel of the chromatogram and the right channel of the method. If they are not compatible (for example if the spectrum channel has been chosen for the chromatogram and an extracted channel for the method) a message will be displayed in the bottom of the screen as follows:



Note: If the chromatogram already contains extracted channels and is reprocessed with a method which contains other extracted channels, all the channels will be added.

If the chromatogram and the method used for reprocessing contain the same extracted channel, the chromatogram will be modified according to the method.

Reprocessing Multiple Chromatograms (Reprocessing List)

To quickly reprocess multiple chromatograms, it is possible to create a reprocessing list:

Select the menu FILE / NEW / NEW REPROCESSING LIST. (Refer to the CompassCDS User Guide to learn how to create a reprocessing list).

The columns of the reprocessing list are very similar to the column of the sequence:

In the “Chromatogram name” column, select the name of the chromatogram(s) to be reprocessed.

In the “Chromatogram channel” column, select the chromatogram channels that should be processed. For example, to reprocess one of two channels, select in this box the name of the channel to reprocess.

In the Method name, select the name of the method to apply. In the method channel, select which method channel should be applied if the method is defined for more than one channel.

Note: If the chromatogram already contains extracted channels and is reprocessed with a method which contains other extracted channels, all the channels will be added.

If the chromatogram and the method used for reprocessing contain the same extracted channel, the chromatogram will be modified according to the method.

Index

3D method, 9
3D variables, 12
Acquisition, 14
Baseline monitoring, 14
Chromatogram, 58
Chromatogram extraction, 10
Extracted chromatogram, 12
Label, 39, 46
Maximum absorbance chromatogram, 18, 36
PDA displaying, 26
Peak purity
  Parameters, 54
  Results, 57
Ratio chromatogram, 58
Reprocess, 62
Spectral library
  New, 41
  Open, 43
  Search, 48